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CONTENTS

Page 313—As the Editor's Note indicates, neither the edited manuscript nor the proof of this paper was seen by the author, and in view of this unusual situation, the editors assumed responsibility for possible misinterpretations of the author's original version. After seeing the printed paper, the author requested publication of the following comments:

Page 314, lines 3 to 5—The natural vegetation of the flooded tirs is not merely an association, but consists of three successive stages, as follows:

1. A consocies of *Phragmites communis*, vegetation very frequently encountered on the tirs that remain submerged a large part of the year.

2. An associes of *Juncus acutus, J. maritimus*, and *Scirpus Holoschoenus*, on the tirs that is still waterlogged but not necessarily submerged.

3. A prairie of *Scirpus maritimus* in the very humid state, as transition to that of moist prairie of Graminaceae and Papilionaceae.

Page 316, table 2, and *page 319*, table 6—*Red sandy soils of the downs* should read as in the original version, *downy red sands* or *downy red sandy soil*. The author points out: "It is not the same. I have created this soil group under the French name 'sables rouges dunaires' or 'série dunaire.' They are soils which, in North Africa, occupy great extents, and are formed, not only on the downs, but also (and more extensively) on the materials of ancient downs and of their soils, which have been removed by erosion and transported by natural agents, as wind, colluvium, etc."

BORON SYMPOSIUM

A symposium on boron was held by the Division of Fertilizer Chemistry of the American Chemical Society at its annual meeting in Pittsburgh, Pennsylvania, on Monday, September 6, 1943, and the symposium papers constitute this issue of SOIL SCIENCE. The widespread interest in boron on the part of pathologists and biological chemists, as well as by those who are concerned with the manufacture and use of fertilizers containing it, resulted in a large attendance at this session. We are pleased to present these papers for the benefit of the readers of this Journal.

<div align="right">FIRMAN E. BEAR</div>

POTASSIUM-BORON AND CALCIUM-BORON RELATIONSHIPS IN PLANT NUTRITION[1]

ELDROW REEVE AND JOHN W. SHIVE[2]

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In recent years the attention of many investigators has centered upon the role of boron in plant nutrition. At present there is much interest in the importance of boron in the commercial production of plants. There is conclusive evidence that many soils are deficient in boron and will not produce a satisfactory crop under any system of soil management unless this element is supplied. Many investigators (3, 8, 10, 12, 14) have observed, in connection with fertilizer experiments on boron-deficient soils, that the severity of boron-deficiency symptoms is often associated with liming of the soil and certain other fertilizer practices. It has been observed frequently also that the external symptoms of boron deficiency and of calcium deficiency are strikingly similar. This has led to the suggestion that the functions of boron and calcium in the plant are intimately associated in the general metabolic activities.

There is some evidence (11) that potassium and boron also are closely related in their effects upon plant development, although this is not indicated by any similarity between the external symptoms induced by deficiencies of these two elements. Little is known, however, about the influence of the major essential cations, K^+, Ca^{++}, and Mg^{++} upon the response of the plant toward boron when these elements are present in the nutrient substrate, or in the plant, either in deficient quantities or in concentrations in excess of those required for growth and development under optimum conditions.

The following report deals primarily with the results of a careful quantitative investigation of the response of plants toward boron treatments when grown separately in series within relatively wide ranges of known levels of potassium and of calcium supplied in a basic nutrient solution in sand culture.

METHOD

Cultural methods

Tomatoes and corn were used as indicator plants in this investigation, since they represent the two distinct types, dicots and monocots, and since earlier work has shown them to be quite different in their response to boron treatment. This report, however, will deal only with the results obtained with the tomato as the indicator plant.

The plants were grown in sand culture, and the nutrient solution was supplied by the continuous flow method (13). Seedlings were germinated in sand and were transplanted to purified sand in highly glazed pots as soon as the cotyledons were free from the seed coat and well expanded, the first true leaves still being in the bud stage.

For 3 weeks after transplanting, all cultures were supplied with a standard culture solution, containing 0.25 p.p.m. boron. The potassium-boron and calcium-boron treatments were begun at the end of this period and were maintained for 14 days.

At the end of the experimental period of 14 days, the plants were harvested, green and dry weight yields were obtained, and the plant material was prepared for the quantitative determinations of boron, calcium, and potassium. In an attempt to differentiate between the total and soluble fraction of each of the three elements, a representative 50-gm. green sample from each culture was quickly frozen at the time of harvest. A 100-gm. green sample from each culture was dried in preparation for the determination of total boron, calcium, and potassium.

Analytical methods

In the presentation of the analytical data it is here assumed that the active fractions of boron, calcium, and potassium in the plant are those portions of each which are in the soluble state within the cells. These fractions were extracted as completely as possible from the frozen samples under pressure. The portions of the elements in question remaining in the press cake are here regarded as the inactive or insoluble fraction.

The frozen tissue was extracted by wrapping the sample in a convenient square of muslin, placing it in the pressure chamber of a Carver press, covering it with 40 ml. of distilled water, and subjecting it to a pressure of 5,000 pounds per square inch for 1 minute. The press cake was washed twice more with 40-ml. portions of distilled water and subjected to 5,000 pounds pressure after each washing. Pressure was maintained for 2 minutes after the last washing. The combined washings were passed through a quantitative filter paper. The press cake was removed from the muslin, placed in a drying basket, together with the filter

paper, and dried at 70°C. for 48 hours. The press cake and filter paper were then ignited in a muffle furnace. The residue was taken up in 10 ml. of (1 + 4) HCl, and made to volume with distilled water in a 250-ml. volumetric flask. Aliquots of this solution were then taken for boron and calcium determinations. All of the potassium was considered to be in a soluble form, since analysis on a number of the press cakes showed that 92 to 99.6 per cent of the potassium in the plant tissues was extractable by the method employed.

Dry yields of the 100-gm. green samples were obtained by drying at 70°C. for 48 hours. The dried tissue was ground in a Wiley mill to pass a 40-mesh screen. A 2-gm. sample was then ignited in a muffle furnace, the residue taken up in 5 ml. of (1 + 4) HCl, and made to volume with distilled water in a 100-ml. volumetric flask. Aliquots of this solution were then taken for the determinations of total boron and total calcium. A separate ashing was made for the potassium determination, and the residue taken up with (1 + 4) HNO_3 instead of HCl, since chlorides interfere in the potassium method here used. Potassium was determined by the micromethod (4).

Calcium determinations were made by the volumetric method of the A.O.A.C. The quinalizarin method (2) was used for the boron analyses.

The soluble fraction of calcium and of boron for each culture was determined by subtracting the total quantity obtained by analysis of the entire residue of an extracted sample from the corresponding total quantity obtained by analysis of an unextracted sample.

POTASSIUM-BORON SERIES

Experimental plan

In this series with tomatoes, twenty cultures, each containing three plants, were divided into five groups of four cultures each. Each group was grown at a different level of potassium. The five potassium levels were: 10, 50, 89, 250, and 500 p.p.m. Boron as boric acid was added at different concentrations to the four cultures at each of the potassium levels. The boron concentrations were as follows: 0.001, 0.1, 0.5, and 5.0 p.p.m. The composition of the culture solutions at the five potassium levels employed with each of the four boron concentrations is presented in table 1.

Response of plants to treatment

The relative intensities of the external symptoms of deficiency and of toxicity which developed on the plants at the different potassium and boron levels are represented by the shaded areas in figure 1. The diagram illustrates also the general experimental setup.

Definite boron-deficiency symptoms developed on the tomato plants in culture 5, supplied with 0.001 p.p.m. boron and 500 p.p.m. potassium, only 3 days after they were placed on treatment. Deficiency symptoms appeared last on the plants of culture 1, supplied with 0.001 p.p.m. boron and at the lowest potassium level, 10 p.p.m. A rough measure of the relative injury to the plants in the different cultures is indicated by the shaded areas in the squares. As shown

in the diagram by the shaded area on the left the severity of the boron-deficiency symptoms on the plants supplied with 0.001 p.p.m. boron in the nutrient solution increased progressively with increase in the potassium concentration in the substrate. It is thus clear that potassium, particularly at the higher concentrations, accentuates the injury resulting from boron deficiency. It also suggests that plants growing in substrates (soils) which are relatively low in boron might be free from symptoms of boron deficiency at low potassium levels, but

TABLE 1

Composition of culture solutions at five potassium levels employed with each of four different boron concentrations of 0.001, 0.1, 0.5, and 5.0 p.p.m.

K LEVELS	MOLAR CONCENTRATIONS OF MAJOR SALTS							TRACE ELEMENTS		
	$Ca(NO_3)_2$	$CaCl_2$	$MgSO_4$	$Mg(H_2PO_4)_2$	KH_2PO_4	KNO_3	K_2SO_4	Fe	Zn	Mn
p.p.m.								*p.p.m.*	*p.p.m.*	*p.p.m.*
10	0.00390	....	0.00182	0.00043	0.00025	....	....	1.0	0.5	0.25
50	0.00382	0.00008	0.00225	....	0.00112	0.00016	....	1.0	0.5	0.25
89	0.00331	0.00059	0.00225	....	0.00112	0.00118	....	1.0	0.5	0.25
250	0.00252	0.00138	0.00225	....	0.00112	0.00265	0.00132	1.0	0.5	0.25
500	0.00252	0.00138	0.00225	....	0.00112	0.00265	0.00453	1.0	0.5	0.25
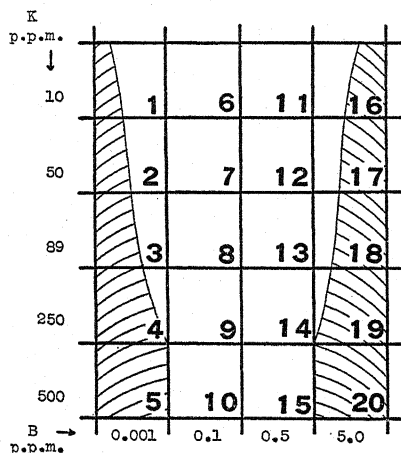
FIG. 1. BORON AND POTASSIUM TREATMENTS AND GENERAL EXPERIMENTAL SETUP FOR POTASSIUM-BORON SERIES

Shaded areas left, and right indicate boron deficiency and boron toxicity, respectively

might be severely injured at the higher levels of potassium. Thus in boron-deficient substrates severe injury may be expected when potassium is present in relatively high concentration, particularly when present in excess of that required by the plant for optimum growth.

At the end of the 14-day treatment period boron toxicity was apparent on all of the plants that were supplied with a solution containing 5.0 p.p.m. boron. But the toxicity on the plants supplied with this relatively high concentration

of boron increased in severity with increase in potassium concentrations in the substrate, as indicated in figure 1 by the shaded areas in the squares representing cultures 16 to 20 inclusive. It is apparent that the external symptoms of boron toxicity at high boron levels, like deficiency symptoms at low boron levels, are progressively accentuated with increasing potassium concentrations in the nutrient substrate.

As indicated in figure 1, no symptoms of either boron toxicity or boron deficiency occurred at any potassium level here used within the range of boron concentrations of 0.1 to 0.5 p.p.m. Within this range, all cultures produced vigorous, healthy plants, and the several cultures showed no pronounced differences in dryweight yields.

Results of chemical analyses

The results of quantitative tests for total and soluble boron in the tissue of the tomato plants are shown graphically in figure 2, in which the boron content of the plants, in parts per million of dry tissue, is plotted against potassium concentration, in parts per million of the substrate. The four sets of graphs represent the analytical data obtained by analyses of the plants grown at four different boron levels.

These graphs bring out the important fact that the potassium concentration of the substrate has a very definite influence on the accumulation of boron in the tissues of the tomato plants. For any given boron concentration in the substrate there is a progressive increase in the boron content of the plants as the potassium concentration in the substrate increases. This is especially pronounced at the high boron levels. For example, at the boron level of 5.0 p.p.m. in the substrate the boron in the plant tissue increased approximately 100 per cent, as the potassium concentration in the substrate was increased from 10 to 250 p.p.m.

These data explain the qualitative observations made earlier, that boron toxicity at high boron levels increased in severity with increase in potassium concentration in the substrate, but fails to explain why injury due to boron deficiency is progressively intensified with increasing concentrations of potassium in the growth medium. In other words, the quantitative data obtained by analysis of the plant tissues for boron and potassium are not in agreement with the qualitative observations made on boron-deficient plants, which suggests that the apparent relation between potassium and boron in the metabolism of the plant is not a direct one but that potassium influences boron nutrition indirectly through some factor which bears a direct relation to boron metabolism. From an analysis of the graphs it appears that the soluble boron in the tissues is a function of the total boron content.

In figure 3 the potassium content of the plant, in milligrams per gram of dry tissue, at each of the five potassium levels is plotted against boron concentration in parts per million of the substrate. As might be expected, the potassium content of the tissues is a function of the potassium concentration in the growth medium. This is indicated by the relative positions of the graphs, one above the other in the order of increasing concentrations of potassium. The outstand-

ing point brought out by these data, however, is the fact that, at each potassium level, except at the lowest, maximum potassium accumulation occurred at a boron concentration of 0.1 p.p.m. in the substate. It will be observed that potassium accumulation increased, from a minimum at 0.001 p.p.m. boron in the substate, to the maximum and then decreased from this point as the boron was increased to 5.0 p.p.m. At the potassium level of 10 p.p.m. in the substrate, however, potassium accumulation was greatest at the lowest boron level (0.001
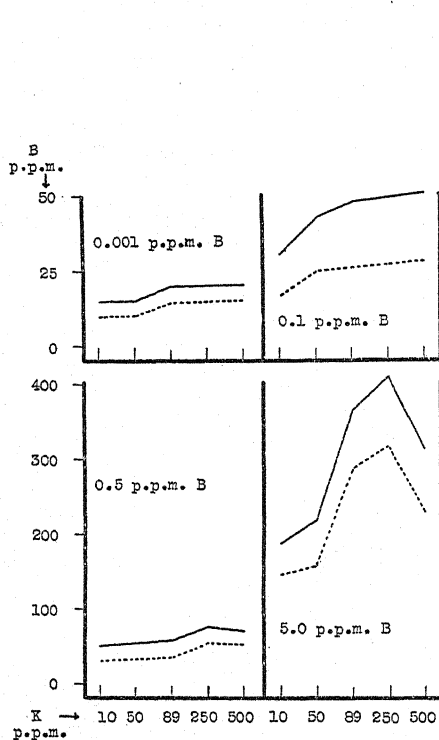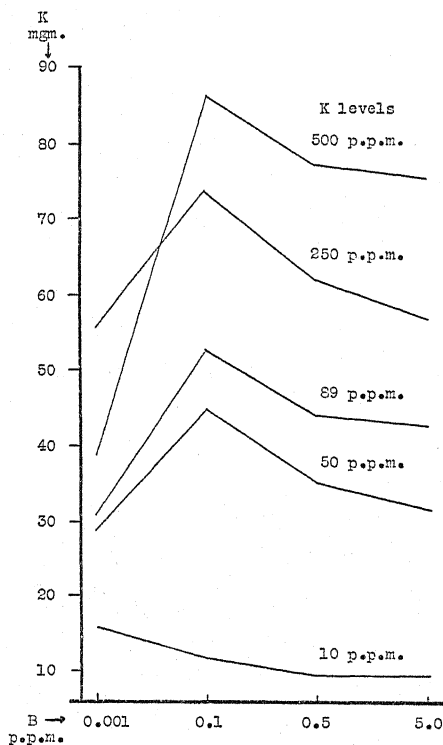
FIG. 2. TOTAL AND SOLUBLE BORON IN DRY PLANT TISSUE AT EACH BORON CONCENTRATION
IN THE SUBSTRATE PLOTTED AGAINST POTASSIUM LEVELS IN THE SUBSTRATE
Solid line, total boron; broken line, soluble boron

FIG. 3. POTASSIUM PER GRAM OF DRY PLANT TISSUE AT EACH POTASSIUM LEVEL IN THE
SUBSTRATE PLOTTED AGAINST BORON CONCENTRATION IN THE SUBSTRATE

p.p.m.) and decreased gradually as the boron concentration in the substrate increased.

The chemical analyses show also that in the tomato plant a considerable portion of the boron which accumulates in the tissues is in the insoluble state. It is logical to assume that boron in this form in the tissues is biologically inactive and its accumulation in this form accounts for the fact that an adequate continuous supply of the element in the nutrient substrate is essential to maintain

normal development at any physiological phase in the growth cycle. The data further show that the greatest average accumulation of inactivated boron occurs in the tissues of the plants which show the highest yields of plant material, that is, where biological activity is at its maximum. These are the plants which were grown at a boron level of 0.10 p.p.m.

<div align="center">CALCIUM-BORON SERIES</div>

Experimental plan

The cultures of this series were carried out in precisely the same way as were those of the potassium-boron series. Thirty cultures, each containing three plants, were divided into six groups of five cultures each. Six levels of calcium were employed: 5, 10, 50, 100, 250, and 500 p.p.m. corresponding to the six groups of cultures. Boron was added to the cultures at each of the calcium levels in concentrations as follows: 0.001, 0.01, 0.5, 5.0, and 10.0 p.p.m. The composition of the culture solutions at the calcium levels employed with each of the boron concentrations is presented in table 2.

Response of plants to treatment

The relative intensities of the symptoms of metabolic disturbance resulting from deficient and from excess quantities of boron in the nutrient solutions supplied to the cultures are represented diagrammatically by the shaded areas in figure 4, which corresponds in every way to figure 1 of the potassium-boron series already treated.

Symptoms of boron deficiency appeared first in the plants of culture 6, at the highest calcium level, 2 days after treatments were begun. At the end of the experimental period the plants of all the cultures at the lowest boron concentration, 0.001 p.p.m., exhibited boron-deficiency symptoms. At the high calcium levels the plants were severely injured, whereas at the lowest calcium level the plants were only slightly injured. At this boron level the deficiency symptoms increased in severity progressively in the order of increasing calcium concentrations of the substrate. This behavior is illustrated in figure 4, on the left, by the shaded area of each square, in proportion to the whole, which roughly represents a measure of the relative degree of injury to the plants resulting from boron deficiency.

A comparison of the diagram of figure 4 with that of figure 1 shows marked agreement between the corresponding areas representing injury from boron deficiency. This indicates that calcium and potassium are similar in their capacity to accentuate the symptoms of boron deficiency with increasing concentrations of these cations in the nutrient substrate. In this respect, however, calcium appears to be considerably more effective than potassium.

Boron toxicity occurred on the plants of all the cultures supplied with a solution containing the highest concentration of boron (10 p.p.m.). As shown in figure 4, however, this type of injury decreased with increasing concentrations of calcium, toxicity being negligible at the highest calcium level. This effect of calcium

is directly opposite to its influence upon the injury produced by boron deficiency and in this respect the influence of calcium is also diametrically opposite to the accentuating effect of potassium on the injury produced by either a deficiency or an excess of boron in the nutrient substrate. This is brought out clearly by a comparison of figure 1 with figure 4.

TABLE 2

Composition of culture solutions at six calcium levels employed with each of five different boron concentrations of 0.001, 0.01, 0.50, 5.0, and 10.0 p.p.m.

Ca LEVELS	MOLAR CONCENTRATIONS OF MAJOR SALTS						TRACE ELEMENTS		
	$Ca(NO_3)_2$	$CaCl_2$	$MgSO_4$	KH_2PO_4	K_2SO_4	$NaNO_3$	Fe	Zn	Mn
p.p.m.							p.p.m.	p.p.m.	p.p.m.
5	0.000125	....	0.001125	0.001125	0.001125	0.00875	1.0	0.5	0.25
10	0.00025	....	0.001125	0.001125	0.001125	0.0085	1.0	0.5	0.25
50	0.00125	....	0.001125	0.001125	0.001125	0.0065	1.0	0.5	0.25
100	0.0025	....	0.001125	0.001125	0.001125	0.0040	1.0	0.5	0.25
250	0.0045	0.00175	0.001125	0.001125	0.001125	....	1.0	0.5	0.25
500	0.0045	0.0080	0.001125	0.001125	0.001125	....	1.0	0.5	0.25
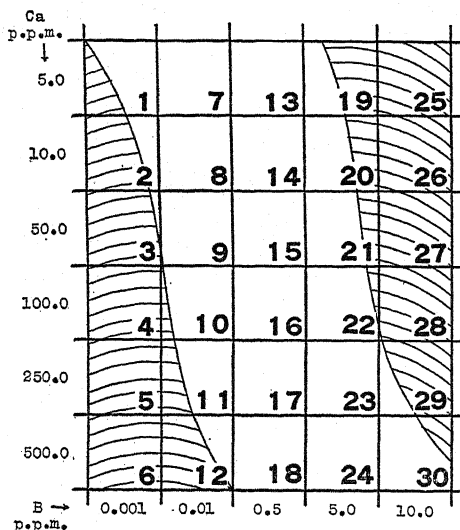
FIG. 4. BORON AND CALCIUM TREATMENTS AND GENERAL EXPERIMENTAL SETUP FOR CALCIUM-BORON SERIES

Shaded areas left and right indicate boron deficiency and boron toxicity, respectively

From the practical point of view, these results suggest that in substrates (soils) which contain boron in excess of that required for optimum growth and development, toxic effects may be reduced or prevented by the addition of calcium. The addition of potassium, on the other hand, should be expected to aggravate the toxic effect of excess boron.

Results of chemical analyses

The results of quantitative tests for total and soluble boron and calcium are given in table 3. An analysis of these data immediately shows that both total

TABLE 3

Total and soluble boron and calcium of tomato plant tissues

Results per gram dry tissue

TREATMENT		TOTAL B	SOLUBLE B	TOTAL Ca	SOLUBLE Ca
Ca	B				
p.p.m.	*p.p.m.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5	0.001	0.018	0.006	9.9	5.0
10	0.001	0.018	0.006	12.0	6.0
50	0.001	0.018	0.006	21.8	8.5
100	0.001	0.018	0.006	25.4	10.2
250	0.001	0.018	0.007	34.6	16.1
500	0.001	0.018	0.005	34.6	15.4
5	0.01	0.025	0.008	9.4	7.5
10	0.01	0.025	0.007	9.2	3.3
50	0.01	0.025	0.009	21.7	9.3
100	0.01	0.025	0.006	24.7	11.4
250	0.01	0.025	0.006	32.7	15.8
500	0.01	0.025	0.006	34.4	20.3
5	0.5	0.062	0.042	6.8	2.5
10	0.5	0.062	0.044	8.2	3.8
50	0.5	0.062	0.041	22.3	8.5
100	0.5	0.062	0.043	23.6	11.4
250	0.5	0.062	0.044	30.3	14.4
500	0.5	0.062	0.042	35.6	17.4
5	5.0	0.468	0.387	6.8	2.7
10	5.0	0.484	0.395	8.1	2.8
50	5.0	0.546	0.386	21.6	7.6
100	5.0	0.429	0.343	23.5	8.5
250	5.0	0.341	0.283	34.7	18.4
500	5.0	0.195	0.136	39.1	19.7
5	10.0	0.859	0.716	7.0	2.7
10	10.0	0.937	0.806	8.7	3.2
50	10.0	1.015	0.727	21.7	6.6
100	10.0	0.693	0.538	23.4	7.3
250	10.0	0.546	0.417	31.5	12.8
500	10.0	0.281	0.211	37.5	16.6

and soluble boron in the plant, at the three lower boron levels, appear to be quite independent of the calcium concentration in the substrate. At the high boron levels (5.0 p.p.m. and 10 p.p.m.), however, there was a decrease in both total and soluble boron concentrations in the tissue, with increase in the calcium concen-

trations in the nutrient substrate. This explains the qualitative observations made earlier, that boron toxicity at the high boron levels decreased in severity with increase in calcium in the nutrient substrate.

It must be concluded from these data that soluble boron in the plant is a function of the total boron content of the tissues, which in turn is determined by the boron concentration in the substrate, but within the limits here defined, boron accumulation in the plant is considerably modified by calcium concentrations in the substrate particularly at higher calcium levels as above indicated. A similar behavior was reported earlier by Marsh and Shive (6) and by Marsh (7).

From the results of the analyses of the plants for total and soluble calcium as presented in table 3, it is clear that calcium accumulation in the tissues is largely determined by the calcium concentration in the substrate and is independent of boron. At the two lower levels of calcium there was a tendency toward slight decrease in total and soluble calcium in the plant with increase in the boron concentration of the substrate. There appears to be little regularity in this tendency, however, and it is probably not significant.

The quantitative data obtained by chemical analyses of the plant tissues may now be considered from another point of view. From the analytical data relating to potassium and boron content of the plants, the K/B ratios for the plants grown at each of the several boron levels have been calculated. These ratios calculated for the plants of the cultures, at a single boron level (0.1 p.p.m.), have been plotted against the potassium concentration of the corresponding culture solutions and are shown in figure 5, which presents in comparison the graph of the Ca/B ratios, similarly calculated for the plants of the same cultures. Since the corresponding ratio curves representing the plants grown at each of the other boron levels are in every respect similar to those presented in figure 5, or at least show similar trends, they will not be considered in this discussion.

As the K/B ratio curve (fig. 5) shows, and as might be expected, the values of the K/B ratio increase rapidly with increase in the potassium concentration of the growth medium. But the interesting and significant fact to be noted is that at any given calcium and boron level, within limits, the Ca/B ratio decreases markedly with increase in the potassium concentration of the nutrient substrate. This indicates the strong inhibitory influence which potassium exercises over the processes controlling the absorption and accumulation of calcium by the plant. It provides further explanation for the marked increase in the severity of boron toxicity at high boron levels with increasing concentrations of potassium, since as has already been shown, the severity of this type of injury increased in intensity as the calcium concentration in the nutrient substrate decreased and, therefore, as the calcium accumulations in the plant tissues decreased. It fails to explain, however, why injury due to boron deficiency is progressively intensified with increasing concentrations of potassium in the growth medium. It is clear that the quantitative data obtained by chemical analysis of the plant tissues for the elements in question are in perfect agreement with qualitative observations based on the severity of the injury produced by toxic quantities of boron, but there is no agreement between the quantitative analytical data and qualitative

observation of the plants based on injury produced by boron deficiency, as already pointed out. The analytical data suggest, however, that the apparent influence of potassium upon the response of the plant toward boron is not a direct relation between potassium and boron in the metabolism of the plant. The response of the plant toward boron appears to be determined by the direct and intimate relation between calcium and boron in metabolism, but potassium strongly influences the response of the plant to boron indirectly through its determinative effects upon the processes involved in the absorption and accumulation of calcium.
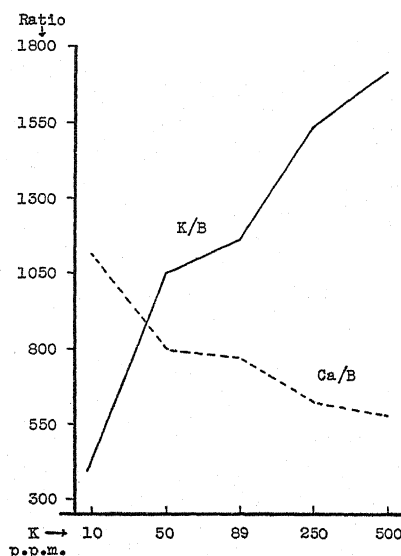
FIG. 5. POTASSIUM-BORON AND CALCIUM-BORON RATIOS IN TOMATO PLANT TISSUE PLOTTED AGAINST POTASSIUM CONCENTRATIONS IN THE SUBSTRATE
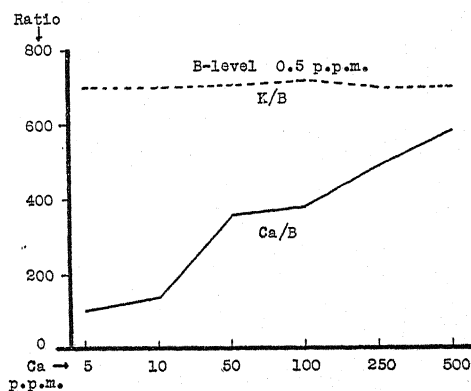
FIG. 6. CALCIUM-BORON AND POTASSIUM-BORON RATIOS IN TOMATO PLANT TISSUE PLOTTED AGAINST CALCIUM CONCENTRATIONS IN THE SUBSTRATE

In figure 6 are presented the Ca/B ratios and the K/B ratios plotted against calcium concentrations in the nutrient substrate in a manner similar to the presentation in figure 5. Here with calcium, as with potassium, the most important single condition concerned with the marked increase in the Ca/B ratio, at constant boron and constant potassium levels, is the absolute concentration of calcium in the growth medium. It is at once obvious, however, from an examination of the graphs, that calcium in the substrate, within the limits of concentrations here employed, has little or no significant effect upon the K/B ratio values. This is in strong contrast to the effect which potassium concentrations in the substrate exert upon the Ca/B ratios (fig. 5). These relations may readily be explained on the basis of the recognized physicochemical properties of the potassium and calcium ions. It is now well known that the potassium ion, being

much more mobile and more active than the calcium ion, tends to retard the processes which determine the absorption and accumulation of calcium (1, 5, 9), and since the calcium ion is less active than that of potassium its influence upon the absorption and accumulation of potassium is secondary, except perhaps at extremely high calcium concentrations.

It is apparent from visible plant response to calcium-boron treatments that the relationships between these two elements are intimate and direct in the nutrition of the plant. This generalization is supported by the fact that qualitative observations of the development of pathological symptoms of both boron deficiency and toxicity are in every instance in perfect agreement with quantitative analytical data and can be explained on this basis. The qualitative observation that potassium has a strong accentuating influence upon boron toxicity in the plant, particularly at potassium concentrations in excess of that required for optimum growth and development, is also in agreement with quantitative evidence, but the observed accelerating effects of potassium upon the injury resulting from boron deficiency is not in agreement with quantitative analytical data. Quantitative experimental evidence to explain this anomaly is at present not available.

The practical aspects of the foregoing considerations may be stated very simply as follows: If a soil on which tomatoes are growing is so deficient in boron that symptoms of deficiency appear on the plants, the application of potassium to the soil would tend strongly to intensify the injury to the plants. This result has been observed both in the field and in artificial cultures under controlled experimental conditions. Here the qualitative observation, which is real, is not in agreement with quantitative experimental evidence, since the boron content of the plant increases correspondingly with increased application of potash to the growth medium, which should reduce this type of injury, not intensify it. This discrepancy between qualitative observation and quantitative experimental evidence is not explainable on the basis of the known physicochemical properties of the ions involved, nor is it in harmony with modern theories of salt antagonisms and cell permeabilities, which, however, fully substantiate the quantitative experimental evidence.

The application of lime to this same boron-deficient soil would unquestionably magnify the injury sustained by the plants, the degree of injury depending upon the size of the application. These results also have been observed in the field and in controlled experimental culture. In this case the qualitative observation is in perfect agreement with quantitative experimental evidence, and both are in harmony with modern theories of salt antagonisms and cell permeability.

Turning now to the other extreme, let us assume that an agricultural soil has a boron content in excess of that required for good growth and development, so that symptoms of toxicity appear on the plants. The application of potash to such a soil would also intensify the injury to the plants, particularly if the quantity of potash applied should be in excess of that required for maximum yields. This result also has been observed both in the field and in experimental cultures under controlled conditions. Here the qualitative observation is in perfect

agreement with the quantitative experimental evidence, is fully substantiated by the physicochemical properties of the ions involved, and is in harmony with the theories of ion antagonisms and cell permeabilities.

If, on the other hand, lime should be applied to this same soil already containing an excess of boron, the injury sustained by the plants would be diminished, and the degree of diminution would be determined by the size of the application of lime. This is the qualitative observation which agrees perfectly with the quantitative experimental data and is also in agreement with the modern theories of ion antagonisms and cell permeabilities as they relate to the absorption and accumulation of ions in the nutrition of plants.

SUMMARY

A careful quantitative study was made of the responses of tomato plants toward different boron treatments when grown within relatively wide ranges of known levels of potassium and of calcium supplied to the plants in a basic nutrient solution in sand culture. The results may be summarized as follows:

The external symptoms of boron toxicity at high boron levels, like deficiency symptoms at low boron levels, are progressively accentuated with increasing potassium concentrations in the nutrient substrate.

The potassium concentration of the substrate has a definite influence upon the accumulation of boron in the tissues of the tomato plant. At any given boron level in the substrate there is a progressive increase in the boron content of the plants as the potassium concentration in the substrate increases. This is especially pronounced at the high boron levels.

Calcium and potassium are very similar in their capacity to accentuate the symptoms of boron deficiency with increasing concentrations of these cations in the nutrient substrate. Boron toxicity, however, at the high boron levels, decreases markedly with increasing concentrations of calcium. In this respect the influence of calcium is opposite to the accentuating effect of potassium.

At the high boron levels there is a marked decrease in both total and soluble boron in the plant tissue with increase in the calcium concentrations in the nutrient substrate. Calcium accumulation in the tissues is largely determined by the calcium concentration in the substrate and appears to be independent of boron.

At any given calcium and boron level, within limits, the Ca/B ratio decreases markedly with increase in the potassium concentration of the nutrient substrate. Calcium, on the other hand, within the limits here employed, has little or no significant effect upon the K/B ratio values.

The response of the tomato plant toward boron appears to be determined by the direct and intimate relation between calcium and boron in metabolism, but potassium appears to influence the response of the plant to boron indirectly through its determinative effects upon the processes involved in the absorption and accumulation of calcium.

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THE CALCIUM-BORON BALANCE IN PLANTS AS RELATED TO BORON NEEDS[1]

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A number of investigators (2, 4, 5, 9, 12, 13, 14) have reported an apparent relationship between the calcium in the soil and availability of boron to plants. In general, boron starvation occurs more frequently in the humid climates and on overlimed or alkaline soils than on acid soils. The exact nature of this variability in the behavior of boron has not been clearly established, although several possibilities have been suggested by these workers.

Purvis (15) pointed out that two of the greatest difficulties in detecting boron deficiency in soils was in differentiating (a) between the total boron content in the soil and the fraction available to plants, and (b) between the total content found in plants and the fraction necessary for normal growth. Drake, Sieling, and Scarseth (3), in a study of boron behavior in which Turkish tobacco was grown on Norfolk sand in a greenhouse experiment, suggested the possibility of using the ratio of the calcium to boron in the plants as a guide in determining the need of boron fertilization.

This paper reports further investigation dealing with the calcium-boron balances in several crops as related to the boron needs.

PLAN OF INVESTIGATION

Alfalfa, oats, and tobacco were grown on Crosby silt loam in greenhouse pot experiments in the usual manner. The pots were glazed 2-gallon crocks.

The treatments, indicated in the tables and graphs, were designed to give plants that would indicate response or toxicity to boron and indicate the effect of the addition of calcium carbonate. The analysis of the soil used is shown in table 1. The quantity of lime needed to obtain the desired pH values was determined as suggested by Naftel (11). The boron analyses were determined by the method of Berger and Truog (1).

RESULTS

Effect of varying lime and borax applications on growth and composition of alfalfa

The Crosby soil was treated as shown in table 2 and planted with inoculated Grimm alfalfa. Two weeks after emergence, the alfalfa was thinned to five plants per pot. No definite signs of boron deficiency or toxicity were shown on any of the treatments by the three crops of alfalfa grown, but the plants receiving no lime and fertilizers were decidedly less vigorous than those from the treated

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TABLE 1

Chemical analysis of Crosby silt loam used in greenhouse pot studies with boron,
Lafayette, Indiana, 1941

Acre weight of soil calculated at 2,000,000 pounds

CHEMICAL ANALYSIS	SAMPLE 1*			SAMPLE 2*		
	1–7″ deep	13–20″ deep	29–30″ deep	0–7″ deep	13–20″ deep	26–36″ deep
pH................................	5.6	6.1	8.3	5.6	5.0	8.1
Total B......................*p.p.m.*	51.0	48.0	49.0	50.0	47.0	46.0
Available B................*p.p.m.*	0.20	0.30	0.35	0.20	0.20	0.20
Total N....................*lbs./A.*	1952.0	1138.0	758.0	1802.0	1050.0	810.0
Total P_2O_5................*lbs./A.*	1232.0	791.0	1600.0	1407.0	865.0	1252.0
Available P_2O_5, at pH 3.0.....*lbs./A.*	5.7	5.3	0.7	7.1	2.1	1.2
Exchangeable CaO......*lbs./A.*	3037.0	7484.0	10842.0	2235.0	4875.0	11012.0
Exchangeable MgO..........*lbs./A.*	789.0	3615.0	3114.0	580.0	2459.0	3212.0
Exchangeable K_2O..........*lbs./A.*	106.0	231.0	81.0	95.0	235.0	102.0
Total Mn...................*lbs./A.*	2880.0	1562.0	1388.0	4160.0	834.0	1786.0

* Sample 1 is the soil from which the surface layer was taken for the greenhouse experiments. Sample 2 is from an adjacent field.

TABLE 2

Yields of alfalfa from Crosby silt loam in the greenhouse where lime and boron were
the variable factors

Yields in oven-dry weight per pot, 5 plants

TREATMENT NUMBER	TREATMENT*	pH OF SOIL†	ALFALFA YIELDS		
			Crop 1	Crop 2	Crop 3
			gm.	*gm.*	*gm.*
1	Untreated	5.6	2.1	3.8	4.1
2	NPK	5.6	4.9	7.3	7.9
3	NPKL	6.6	6.4	8.0	8.4
4	NPK2L	7.6	6.4	8.3	8.5
5	NPKL + B	6.7	6.0	7.5	7.6
6	NPKL + 2B	6.5	5.7	7.9	8.6
7	NPKL + 3B	6.6	6.5	7.8	8.6
8	NPK2L + B	7.6	6.7	8.2	7.6
9	NPK2L + 2B	7.7	6.9	7.9	8.3
10	NPK2L + 3B	7.7	6.6	7.8	9.3

* NPK = Nitrate of soda, 20 per cent superphosphate, and muriate of potash added in adequate amounts as indicated by tissue tests.

  L = 1800 pounds calcium carbonate per acre
2L = 4000 pounds calcium carbonate per acre
  B =   10 pounds borax per acre
2B =   25 pounds borax per acre
3B =   50 pounds borax per acre
† pH determinations on soil were made 48 days after addition of treatments.

pots. The growth of the alfalfa was not so good on the unlimed pots with a pH of 5.6 as on the limed soil with a pH of 6.6 and 7.6. Varying the borax applications in the two series of limed pots produced no definite changes in the yield.

The variations in the contents of calcium and boron as a result of the treatment were not great, as shown in table 3. The variations are most evident when expressed as a ratio between the calcium and boron. This ratio becomes lower as the applications of borax increase. The figures also show that the ratio was consistently higher with the highest pH values resulting from the lime application.

Since there was no evidence of boron deficiency or boron toxicity in the yields and no symptoms of deficiency or toxicity, the conclusion is reached that the

TABLE 3

Calcium and boron content of alfalfa from greenhouse studies on Crosby silt loam where lime and boron were the variable factors

TREAT-MENT NUMBER	TREATMENT*	CROP 1			CROP 2			CROP 3		
		Ca	B	Ratio Ca/B	Ca	B	Ratio Ca/B	Ca	B	Ratio Ca/B
		p.p.m.	*p.p.m.*		*p.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*	
1	Untreated	17,200	63	273	14,000	45	311	14,500	59	246
2	NPK	13,700	36	380	12,600	42†	276	11,200	38†	266
3	NPKL	14,900	40	372	13,500	38	355	13,600	35	389
4	NPK2L	17,600	30	587	14,400	29	497	16,200	30	537
5	NPKL + B	14,400	63	229	12,100	78	155	12,400	77	161
6	NPKL + 2B	16,200	67	242	13,900	95	146	13,000	107	121
7	NPKL + 3B	15,100	104	145	12,600	121†	104	14,400	183	80
8	NPK2L + B	16,100	46	350	14,400	51	247	14,200	67	212
9	NPK2L + 2B	16,600	59	281	13,100	99	132	14,300	112	128
10	NPK2L + 3B	17,400	87	200	14,600	118	124	14,800	145	102

* See footnote table 2.

† Average analyses from two pots only. All other values shown are averages from triplicates.

calcium-boron ratio between 80 and 587 represents a range in which no deficiencies or toxicities will occur with alfalfa on this soil. These data indicate that relatively high quantities of borax can be added to heavily limed soils without danger of toxicity to alfalfa. The yield was not reduced by liming to pH 7.6, where the alfalfa was grown without borax. This indicates that boron deficiency may not be expected where the calcium-boron ratio is approximately 600. Because of the limited data this is not conclusive, and further tests should be made.

When borax was added to the soil at rates from 10 to 50 pounds per acre the amount of boron taken in by the plant increased in rough proportion to the amount of borax applied. When the soil was limed, however, less boron was taken up at the higher pH values for each increment of borax addition than at the

lower pH values. This may indicate some influence of the excess calcium upon the availability of the boron in the soil.

TABLE 4

Yields and chemical analyses of oats grown on Crosby silt loam in the greenhouse with various fertilizer treatments

TREATMENT NUMBER	TREATMENT*	pH OF SOIL†	DRY WEIGHT OF PLANTS‡	PLANT ANALYSIS		
				Ca	B	Ratio Ca/B
			gm.	p.p.m.	p.p.m.	
1	Untreated	6.3	1.4	7,800	20	390
2	L	7.3	1.7	8,900	17	524
3	NKL	7.0	1.5	9,700	19	510
4	PKL	7.0	3.8	11,700	15	780
5	NPL	·7.0	5.1	11,300	20	565
6	NPK	6.0	7.4	8,600	20	430
7	NPK + B	5.9	6.5	8,500	50	170
8	NPK +2B	6.0	7.0	7,300	97	78
9	NPK +3B	5.8	7.5	7,600	317	24
10	NPK +4B	5.8	6.0	7,000	400+	18§
11	NPK +5B	5.7	4.6	6,800	400+	17§
12	NPKL	7.1	6.2	9,400	17	553
13	NPKL + B	7.1	6.0	9,500	24	400
14	NPKL +2B	7.0	5.9	9,500	54	176
15	NPKL +3B	7.0	6.3	8,400	155	59
16	NPKL +4B	7.0	6.5	7,600	395	19
17	NPKL +5B	7.0	6.4	7,300	400+	18§
18	NPK2L + B	8.2	6.8	11,300	22	514
19	NPK2L +2B	8.2	6.6	12,400	44	282
20	NPK2L +3B	8.0	6.9	11,400	85	134
21	NPK2L +4B	8.2	6.2	10,500	355	30
22	NPK2L +5B	8.2	6.3	11,500	400+	28§

* NPK = Ammonium sulfate, nitrate of soda, 20 per cent superphosphate, and muriate of potash added in adequate amounts as checked by tissue tests.

L = 1700 pounds calcium carbonate per acre
2L = 5000 pounds calcium carbonate per acre
B =   10 pounds borax per acre
2B =   25 pounds borax per acre
3B =   50 pounds borax per acre
4B = 100 pounds borax per acre
5B = 200 pounds borax per acre

† pH determinations were made 72 days after application of treatments.

‡ Yields represent averages of duplicates for treatments. Chemical analyses are from this material.

§ The ratios in these cases are lower than the figures indicate.

Effect of varying lime and borax applications on growth and composition of oats

Following the experiment with the alfalfa, oats were grown on soil taken from the same field. The treatments, yields, and calcium-boron analyses are shown

in table 4.   Between the time of taking the soil for the alfalfa and that for the oats the field had been limed with 900 pounds of ground limestone per acre.

When the oat plants started to head, they were harvested for dry weight determinations and chemical analysis.

Within 3 days after the plants emerged, all those growing on the soil receiving 25 or more pounds of borax per acre showed boron-toxicity symptoms, which

TABLE 5

Yields and chemical analyses of tobacco grown on Crosby silt loam in the greenhouse with various fertilizer treatments

TREATMENT NUMBER	TREATMENT*	pH OF SOIL†	DRY WEIGHT OF PLANTS‡	PLANT ANALYSIS‡		
				Ca	B	Ratio Ca/B
			gm.	p.p.m.	p.p.m.	
1	None	6.2	1.2	31,100	23	1,481
2	L	7.2	1.9	31,100	25	1,382
3	NKL	7.0	1.2	30,800	20	1,502
4	PKL	7.1	4.9	29,000	21	1,381
5	NPL	7.3	9.6	31,200	22	1,387
6	NPK	5.7	13.6	34,700	22	1,542
7	NPK + B	5.7	10.0	23,200	23	1,009
8	NPK +2B	5.7	9.5	27,100	91	300
9	NPK +3B	5.8	7.3	32,200	176	183
10	NPKL	7.2	9.3	33,800	20	1,690
11	NPKL + B	7.1	10.1	29,100	18	1,617
12	NPKL +2B	6.9	10.4	31,900	35	911
13	NPKL +3B	7.1	9.4	29,000	88	330
14	NPK2L + B	7.8	7.8	39,000	22	1,733
15	NPK2L + 2B	8.2	8.6	42,300	32	1,302
16	NPK2L + 3B	8.0	6.4	41,700	44	948

* NPK = Ammonium sulfate, nitrate of soda, 20 per cent superphosphate, and muriate of potash added in adequate amounts as checked by tissue tests.
  L = 1700 pounds calcium carbonate per acre
 2L = 5000 pounds calcium carbonate per acre
  B =   15 pounds borax per acre
 2B =   50 pounds borax per acre
 3B = 100 pounds borax per acre
 † pH determinations were made 72 days after application of treatments.
 ‡ Each value represents an average of duplicates.

were manifested by a dying of the leaf tips.   Where 200 pounds of borax per acre was used without lime about half of the leaf tissue was destroyed, but where the higher application of lime was used the injury was less severe.

The calcium-boron ratios exhibit the same general trend in response to additions of lime or varying amounts of borax to the soil as those of the alfalfa.   When the ratio was lower than 200, however, the plants showed boron toxicity.   The lower the ratio from this value, the greater was the toxic effect.   At a ratio of

approximately 600, the plants were healthy, indicating that boron deficiency would not occur until this ratio was higher.

Effect of varying lime and borax applications on growth and composition of tobacco

Simultaneously with the oat experiment, tobacco was grown on a similar soil. The treatment of the soil, the yield, and the composition of the plants with respect to calcium and boron are shown in table 5.

Three tobacco seedlings were transplanted to each pot. At first the growth was slow and no boron-toxicity symptoms were noticed. The seedlings, however, did not become established on the soils treated with 200 pounds of borax per acre despite repeated transplantings. Where as little as 15 pounds of borax was used without lime, the yield was slightly reduced. This seems to indicate the sensitive nature of tobacco toward borax.

The calcium-boron ratios for the various treatments indicate that when the ratio was less than 1200 the plants were injured by the borax. The lower the ratio from this value, the greater was the toxic effect. In all cases where the ratio was above 1200 the plants were healthy. The yield varied, but as a function of the major nutrient factors.

In an earlier investigation (3) with tobacco the optimum calcium-boron ratio appeared to be approximately 1200. In that investigation, when the ratio was 1500 to 2100 the tobacco plants showed marked boron-deficiency symptoms. This indicates that the boundary between boron deficiency and the optimum ratio would lie somewhere between 1200 and 1500, although at a ratio of 1700 in this investigation boron-deficiency symptoms were not noted.

DISCUSSION

Reeve and Shive (16) found that as corn and tomato plants absorb increasing quantities of potassium, increasing quantities of boron are absorbed, and that the greatest boron toxicity occurred at the highest potassium level. Since the concentration of potassium in plant tissues tends to be reciprocal to that of calcium, the high levels of potassium would be associated with a low intake of calcium (7). Thus, the ratio of calcium to boron would be lower for the particular plant, and the toxic effect would be related more directly to the calcium and only in a secondary sense to the potassium.

Marsh (8) compared the calcium and boron content of two dicots—tobacco and faba beans—with that of two monocots—corn and oats—under varying levels of boron and calcium in sand culture where the other nutrients were adequate. When the calcium-boron ratios of his data are calculated, the relationships as shown in figure 1 are revealed. With "deficient boron and optimum calcium" very high ratios are obtained. It is interesting to note that with "deficient boron" and "deficient calcium" as well as with "optimum boron and optimum calcium" the ratios are very similar within each group of plants. In the case of the monocots our interpretation would suggest with the "deficient boron and deficient calcium" the boron was adequate at the level of performance by the plant and that the calcium was the principal functional determinate. The "toxic

boron-optimum calcium" and "optimum boron-deficient calcium" plants have ratios that approach the border line between healthy and toxic plants in our own investigations. With the "optimum boron-deficient calcium" plants, the question arises as to whether or not this was actually a case of toxic boron instead of "optimum boron." In the dicots, similar relationships appear to exist but at different levels from those of the monocots. The zone of hachuring on figure 1 indicates the boundary between toxic and healthy plants for oats and tobacco as found in the investigations reported here.

The data by Marsh indicate that the calcium-boron ratio can be criticised as an indication of boron needs on the basis that it does not tell whether sufficient
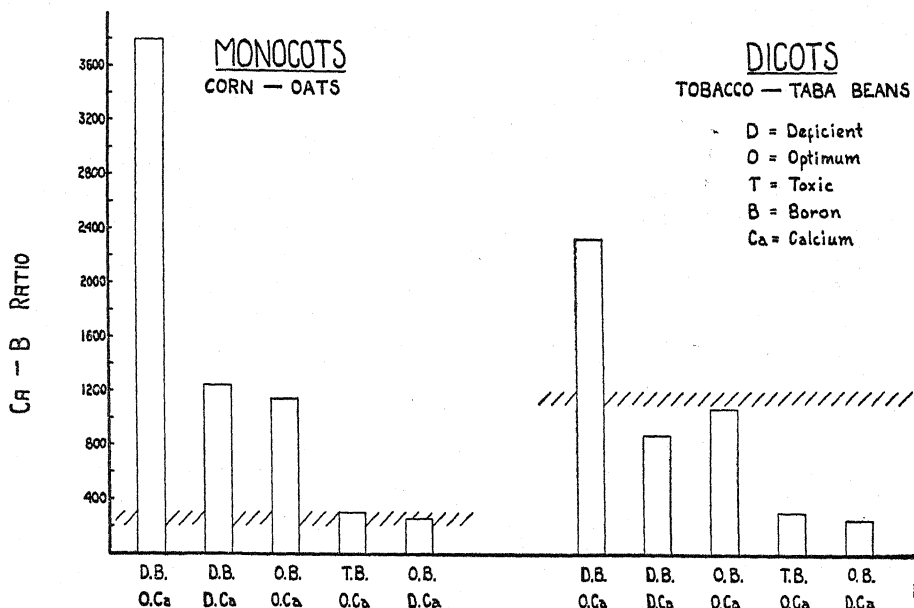
FIG. 1. CALCIUM-BORON RATIOS OF MONOCOTS AND DICOTS FROM EXPERIMENTS CONDUCTED
BY MARSH (8)
The hachured area indicates the approximate limit of boron toxicity

calcium and boron are present for optimum growth even though they are in the proper proportion. It is apparent that the ratio alone does not indicate whether the low ratio is caused by a deficiency of calcium or a toxicity of boron. A knowledge of the requirement of plants for calcium and boron, however, should make it possible to explain questions arising from the above condition.

An effort was made to find data in the literature that could be plotted to determine whether any relationship existed between the yield and the calcium-boron ratio in various crops where deficient, optimum, and toxic conditions existed. Data for sugar beets (2), soybeans (10), and tobacco (3) were found and are plotted in figure 2. These curves are very limited in their value, yet they indicate that plants have a considerable range of tolerance for either deficiency or

excess of available boron in the soils before yields are greatly affected. Once this elastic limit is exceeded the yields are materially decreased and the entire plant may die.

This research indicates that a number of soil and plant factors are of importance in the application of the calcium-boron ratio. The importance of the proper balance of calcium to boron, both in quantity and proportion, cannot be
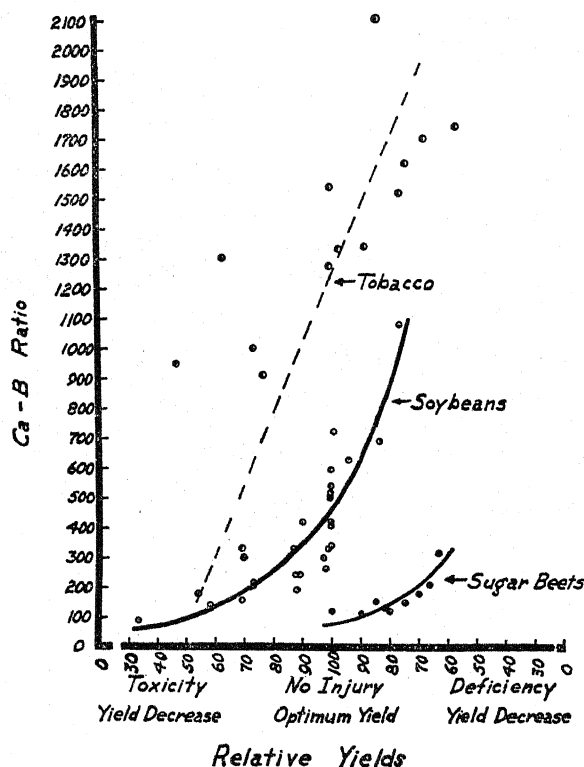
FIG. 2. COMPARISON OF THE CALCIUM-BORON RATIOS OF SUGAR BEETS, SOYBEANS, AND TOBACCO WITH RELATIVE YIELDS ON A NUMBER OF DIFFERENT SOILS AS REPORTED BY VARIOUS WORKERS

Data for the ratios and yields for the sugar beets were taken from Cook and Millar (2) for the soybeans from Muhr (10); and for the tobacco from Drake, Sieling, and Scarseth (3) and from Jones (6).

overemphasized. The factors which affect this balance must be taken into consideration in interpreting any condition in the dynamic-soil relationships. These investigations suggest that it may prove valuable to obtain more analyses on the calcium-boron ratio over a wide range of soils, climates, and fertility for the different kinds of plants. A quantity of such data would have valuable practical application in predicting the boron needs from the analysis of the plant.

SUMMARY

A study was made of several Indiana farm crops in greenhouse pot experiments where borax was added in varying amounts to limed and unlimed soils. The crops were analyzed for calcium and boron. It was found that plants will take up varying quantities of calcium and boron depending upon the availability of these elements in the soil. From plant analyses it appears that each plant has a specific need for calcium and boron, but the range varies greatly for different kinds of crops. The plant will make a normal growth only when a certain balance in the intake of calcium and boron exists. If this balance is upset by a small intake of calcium, such as occurs on acid soils, the plant will have a very low tolerance for boron. On strongly acid soils that contain a small quantity of available calcium, small additions of borax applied to the soil may cause boron injury to the plant.

On soils of the humid region that have a very high calcium content such as alkaline or overlimed soils, the plants require more boron than on the acid soils. On such alkaline soils the balance in the calcium and boron relationship tends to be upset because of the excess calcium the plants have absorbed. On overlimed Indiana soils, the plant may require more boron than is available.

Boron can be added in larger quantities on alkaline or limed soil without causing any injury or toxic effect than when added to acid soils. In practice, this means that farmers should be advised to be on the lookout for boron starvation particularly on overlimed or alkaline soils and should be careful not to add overdoses of borax to acid soils where it may cause injury if added in too high quantities.

The ideal balance between calcium and boron for tobacco appears to be about 1200 of calcium to 1 of boron; this ratio is in terms of equivalent weights of the two elements. For soybeans the ratio of calcium to boron is about 500 and for sugar beets about 100. It appears that this information will be helpful in predicting where more boron is needed as well as to indicate where the application of boron to soils may cause injury.

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BORON TESTS AND DETERMINATION FOR SOILS AND PLANTS[1]

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Since publication of our first report (1) on the quinalizarin method for the determination of boron in soils and plants, certain improvements in the procedure have been made. The primary purpose of the present paper is to describe these. A brief review of the pertinent literature which has appeared since the first report is also given.

McHargue *et al.* (4, 5, 6, 7) have carried on extensive investigations dealing with the advantages and precision of the quinalizarin, titration, curcumin, and spectroscopic methods for the determination of boron in soils and plant materials. In a recent publication (6) they report that the quinalizarin method gives satisfactory results for the determination of boron in plant materials. When the results were compared with those obtained by means of spectrographic analyses, good correlation was obtained. Also (8), carbonate fusion followed by use of quinalizarin colorimetric procedure, essentially as described by Berger and Truog (1), is said to appear to be the most convenient routine method for the determination of total boron in soils. Results obtained with the quinalizarin procedure compared more favorably with those of the official method than did the results with the Naftel curcumin (turmeric) procedure (9).

De Turk and Olson (3) report that the titration procedure (11) apparently does not always give satisfactory results with small amounts of boron.

Maunsell (8) reports satisfactory results when the quinalizarin procedure is used to determine small quantities of boron in soils and plant materials.

PREPARATION OF REAGENTS FOR QUINALIZARIN PROCEDURE

Following our first report (1) it was found to be convenient and also conducive to accuracy to dissolve the quinalizarin directly in 98 per cent by weight H_2SO_4. Previously, separate solutions of 98.5 per cent by weight H_2SO_4 and quinalizarin in 98.5 per cent by weight H_2SO_4 were employed. The use of 10 cc. of the combination reagent with 1 cc. of test solution produces the same final concentration of acid and quinalizarin in the test solution as was obtained formerly with the separate reagents. The combination reagent is simpler to prepare than the two, requires only one storage vessel with protective and dispensing accessories, and contributes to accuracy in measuring the amount of reagent needed per test. It

is noted that McHargue and Hodgkiss (5, 6, 7) have been using a similar combination reagent for some time.

Quinalizarin-sulfuric acid solution—98 per cent by weight H_2SO_4 containing 5 mgm. of quinalizarin per liter. Although the strength of the sulfuric acid in this solution may vary from 97.5 to 98.5 per cent by weight, it is very important to keep it within these limits for small amounts of boron, and this requires accurate work in its preparation and great care in its use and storage. The acid is prepared by mixing ordinary c.p. concentrated sulfuric acid with fuming sulfuric acid. To facilitate the calculations involved in determining the proportion of each to be mixed, strengths are expressed in terms of sulfur trioxide rather than sulfuric acid. Accordingly, the desired 98 per cent sulfuric acid becomes 80.0 per cent sulfur trioxide. The proportion of each to be mixed varies, of course, with the strengths of the acids. The concentrated acid usually contains about 95 per cent of sulfuric acid by weight; and the fuming, 20 to 30 per cent of free sulfur trioxide. The exact strength must be determined for each lot used, because the variation is often great enough to cause serious errors. This is done by weighing out 2 gm. or more of the acid in a 25-cc. weighing bottle, and after diluting, titrating with 1.0 N sodium hydroxide. In the case of the concentrated sulfuric acid, the weighing bottle with contents is placed in a beaker of water, and, after mixing, the acid is titrated. In the case of the fuming acid, the weighing bottle containing the acid is dropped into a second 100-cc. weighing bottle containing about 30 cc. of water. As the bottle is dropped, the cover of the 25-cc. bottle is removed, and then the cover of the larger bottle is quickly replaced. After standing overnight or until fuming has entirely ceased, the two weighing bottles with covers removed are placed in a liter beaker containing 300 to 400 cc. of water. It is advisable to place the cover of the larger bottle in the beaker also, to prevent any loss of acid which might be on the cover. After mixing, the acid is titrated. The strengths in terms of sulfur trioxide are then calculated by means of the following formula:

$$\frac{\text{cc. NaOH titration} \times \text{normality} \times 0.04003}{\text{weight of concentrated or fuming } H_2SO_4} \times 100 = \text{percentage by weight of } SO_3 \text{ in each case}$$

After the strengths of the acids have been determined in terms of sulfur trioxide, the proportion of each to be mixed to make 100 gm. of 98 per cent sulfuric acid, which contains 80.0 per cent of sulfur trioxide, is calculated as follows:

Let x = grams of concentrated sulfuric acid needed
100 − x = grams of fuming sulfuric acid needed
a = strength of concentrated sulfuric acid in terms of sulfur trioxide expressed decimally
b = strength of fuming sulfuric acid in terms of sulfur trioxide expressed decimally.

Then, $ax + b(100 - x) = 80.0$ and x is solved for.

For example: Strength of concentrated sulfuric acid found by titration was 77.78 per cent sulfur trioxide. Strength of fuming sulfuric acid found by titra-

tion was 87.41 per cent sulfur trioxide. Substituting these values in the formula above and solving for x, there is obtained:

$$0.7778x + 0.8741(100 - x) = 80.0$$
$$0.0963x = 7.41$$
$$x = 76.9 \text{ gm., amount of concentrated acid needed}$$
$$100 - 76.9 = 23.1 \text{ gm. of fuming acid needed in making 100 gm. of}$$
desired mixture containing 98.0 per cent H_2SO_4.

To each liter of 98 per cent sulfuric acid, add 5 mgm. of quinalizarin and mix thoroughly to dissolve. Store in a tightly stoppered glass bottle to prevent change in acid concentration, or better, in accordance with the apparatus illus-
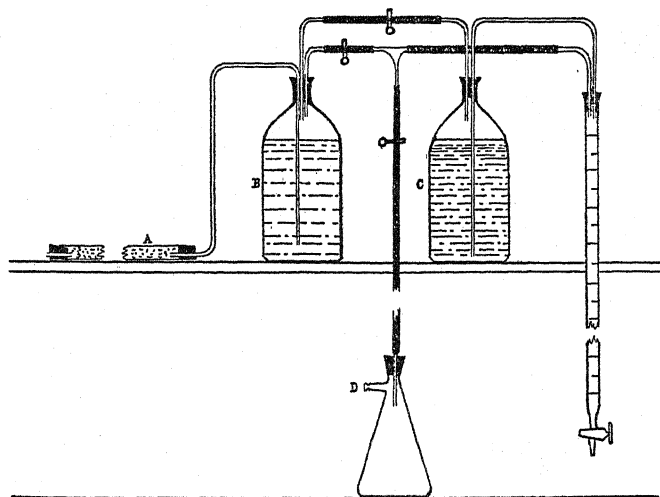
FIG. 1. APPARATUS FOR STORING AND DELIVERING QUINALIZARIN-SULFURIC ACID SOLUTION SO AS TO PREVENT ABSORPTION OF WATER FROM THE AIR

A, Tube containing anhydrous $CaCl_2$; B, Bottle containing 98 per cent by weight H_2SO_4; C, Bottle containing quinalizarin-sulfuric acid solution; and D, Suction flask. To fill burette, apply suction at D and release lower and upper clamps. To dispense solution from burette, release middle clamp.

trated in figure 1, which obviates contact with moist air during storage and facilitates accurate dispensing.

Sulfuric acid, approximately 0.36 N. Dilute 5 cc. of 95 to 96 per cent by weight sulfuric acid to 500 cc. with distilled water.

Sulfuric acid, approximately 4 N. Dilute 50 cc. of 95 to 96 per cent by weight sulfuric acid to 450 cc. with distilled water.

Calcium hydroxide, saturated solution. Add 5 to 10 gm. of calcium hydroxide to 500 cc. of distilled water. Shake well and allow to settle.

Potassium carbonate solution. Dissolve 40 gm. of anhydrous potassium carbonate in 100 cc. of distilled water. Five drops of this contain about 0.1 gm. of potassium carbonate.

Standard boric acid solutions. Dissolve 2.8578 gm. of boric acid in 1000 cc. of

distilled water. This solution contains 0.5 mgm. of boron per cubic centimeter and serves as the primary (A) base stock solution. Prepare a second (B) stock solution containing 0.01 mgm. of boron per cubic centimeter by diluting 20 cc. of the primary base stock solution to 1000 cc. with distilled water, and a third (C) stock solution containing 0.001 mgm. of boron per cubic centimeter by diluting 100 cc. of the second stock solution to 1000 cc.
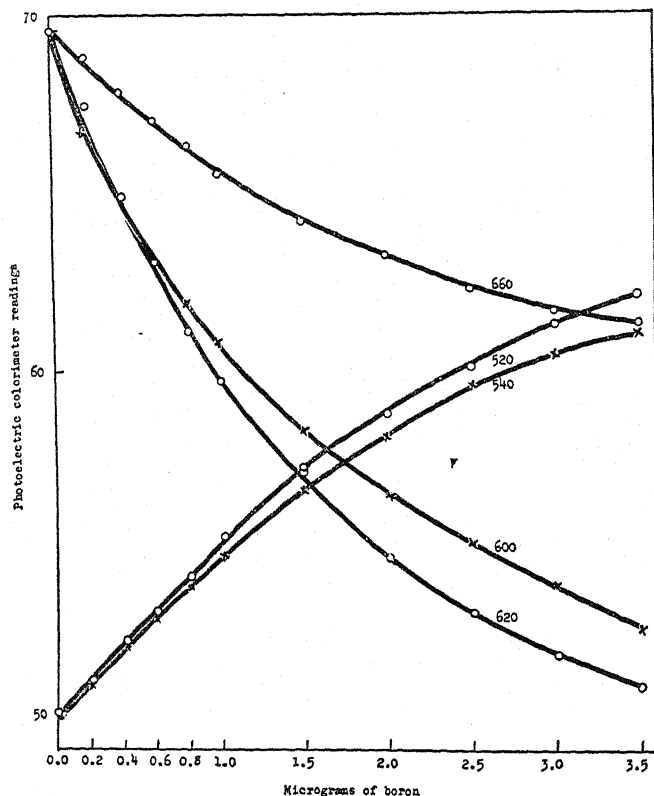
FIG. 2. PHOTOELECTRIC COLORIMETER READINGS AND CURVES OBTAINED IN THE QUINALIZARIN PROCEDURE WITH FILTERS AND AMOUNTS OF BORON INDICATED

USE OF PHOTOELECTRIC COLORIMETER

Although the color comparisons of unknown test solutions with standard color solutions can be made very satisfactorily by visual observation, it was deemed desirable to investigate the use of a photoelectric colorimeter for this purpose. Olson and De Turk (10), and McHargue and Hodgkiss (6, 7) have used photoelectric colorimeters in this connection. In the present investigation an Evelyn photoelectric colorimeter was employed.

Selection of filter

In test solutions to which known amounts of boron were added, the color produced on adding quinalizarin was developed in the regular way as described under

analytical procedure. Colorimeter readings were then made with the filters and standard boron concentrations indicated in figure 2. It will be noted that filter 620 (595 to 660 mμ transmittance) gives the greatest range in scale readings, and this filter was accordingly used in subsequent work.
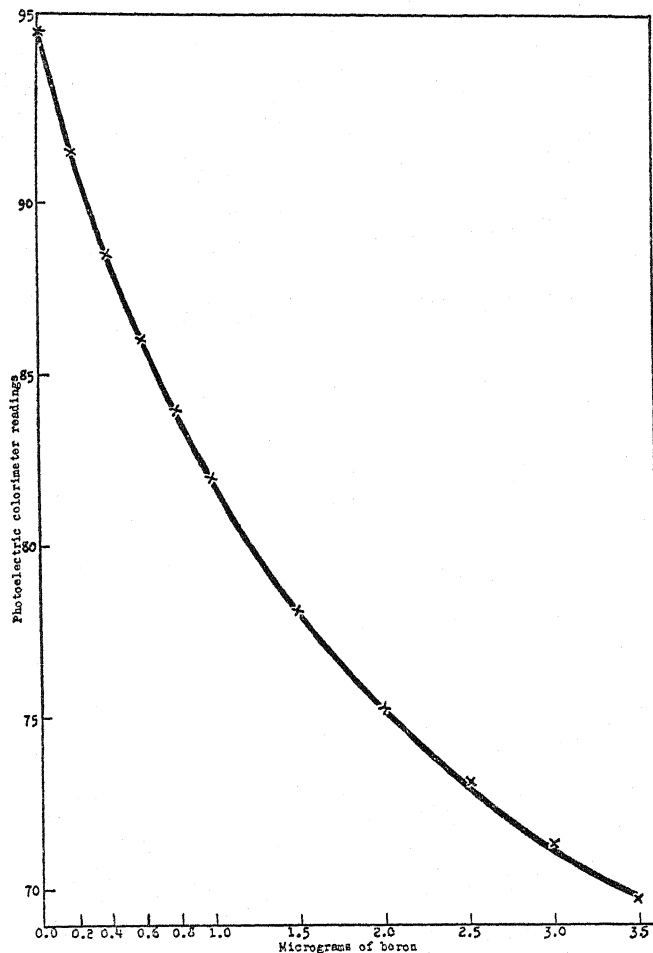
FIG. 3. STANDARD CURVE FOR USE IN THE QUINALIZARIN PROCEDURE AT A TEMPERATURE OF 25°C., WITH A 620μ, FILTER, AND GIVING AMOUNTS OF BORON PRESENT WHEN PHOTOELECTRIC COLORIMETER READINGS ARE AS INDICATED

Standard curve for use with 620 filter

By means of standard color solutions as before, a curve for use with filter 620 was prepared, the zero point being set at a scale reading of 95. A smooth curve representing a wide range of scale readings for a limited range of boron concentrations was obtained (fig. 3). Because of slight changes in acid concentration and colorimeter adjustments from time to time, it is necessary to check the standard curve frequently and make a new one if necessary.

Selection of colorimeter comparison tubes

For holding the test solutions in the Evelyn colorimeter, selected ordinary 7-inch by 7/8-inch test tubes are used. Obviously for boron determinations these tubes must be made of boron-free glass. The high refractive index of the concentrated H_2SO_4 test solution tends to magnify greatly the influence on light transmittance of any small defects or scratches on the comparison tubes. It was found that the best method of selecting a satisfactory set of comparison tubes is to place about 15 cc. of the test solution (the solution of previous tests may be used) in each tube and determine the scale readings of the colorimeter for each tube when a 620 filter is used. Tubes are then selected which give practically the same reading. In many cases the reading changes with a change in orientation of the tube. If tubes showing this characteristic are to be used, it is necessary, by means of a suitable mark, to maintain the same orientation during all readings. Slight variations in tubes may be compensated for by using a correction factor. All tubes should be checked frequently, because any slight scratching or marring that results from handling may seriously influence the transmission of light. The outside of the tubes should be perfectly clean and relatively free of condensed moisture when readings are made.

INFLUENCE OF TEMPERATURE ON SHADE OF COLOR

Olson and De Turk (10) have investigated the influence of temperature on the shade of color produced in the quinalizarin reaction with boron. They noted a considerable color change in the temperature range of 65 to 90°F., and indicate that control is desirable. Maunsell (8) found that the final color is the same after cooling, regardless of the temperature of the solution when the quinalizarin is added.

To obtain more information regarding the influence of temperature on the shade of color produced, readings were taken of test solutions containing different amounts of boron and the regular amounts of acid and quinalizarin given under analytical procedure, at four temperatures, 0, 25, 50, and 85°C. The results obtained are given in figure 4. It will be noted that the readings are influenced greatly by the temperature, and that a smooth curve is obtained at any temperature in the range represented, provided this temperature is maintained throughout a set of readings.

It should be explained that quinalizarin dissolved in strong H_2SO_4 gives a reddish color, and on reacting with boric acid under these conditions, the color changes toward blue. Also, the bluer the test solution, the lower is the colorimeter reading when a 620 filter is used. It will be noted that a more intense blue color was obtained with the lower temperatures. This, however, does not necessarily mean that the reaction between the quinalizarin and boric acid is not complete at the higher temperatures, but rather that there is less distortion of the large quinalizarin-boric acid molecule by the strong sulfuric acid, inasmuch as it is believed that the color change arising from boric acid is due to an increased distortion of the quinalizarin-boric acid molecule over that of the quinalizarin molecule itself.

It is to be noted further that the lower the temperature, the greater is the range of colorimeter readings in going from low to higher concentrations of boron. Because moisture condenses on the comparison tubes when the temperature is markedly below room temperature, it is not convenient to operate at temperatures much below the ordinary room temperature. Since a temperature of 25°C. gives a satisfactory range and is convenient, it was decided to make subsequent readings at this temperature.

Previously the influence of temperature in this connection was not understood, and directions were given to allow the test solution to stand for at least 15 minutes after adding the reagents, in order to obtain full color development. It is
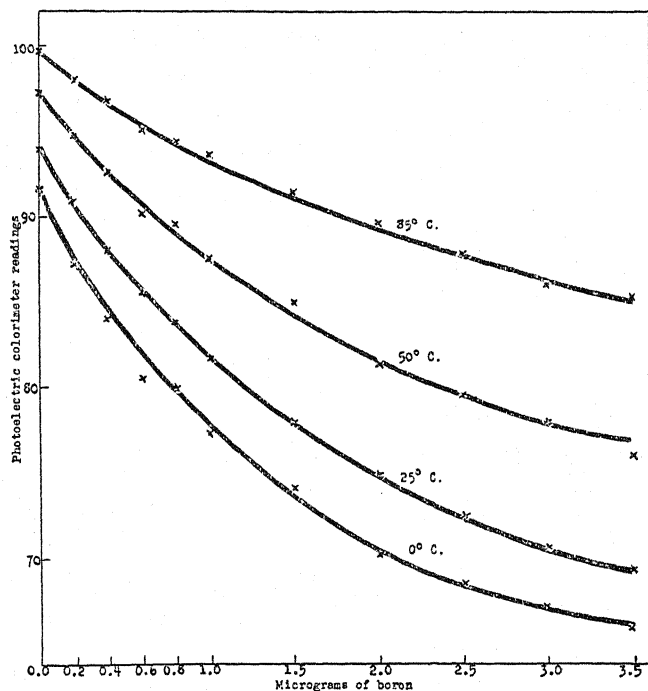
FIG. 4. INFLUENCE OF TEMPERATURE ON PHOTOELECTRIC COLORIMETER READINGS IN QUINALIZARIN PROCEDURE FOR BORON

now known that the color develops as soon as the test solution is cooled to the desired temperature.

ANALYTICAL PROCEDURES

Preparation of color standards for visual comparison

Transfer varying amounts of the stock solution B (for 0.001–0.0025 mgm. of boron) and C (for 0.000–0.001 mgm. of boron) to boron-free test tubes or glass vials. Glass vials approximately 20 by 100 mm. are convenient and satisfactory for this purpose. For the correct concentration of acid when the color is developed, it is necessary to have exactly 1 cc. of boric acid solution in each vial. This

is done conveniently by dispensing the boron stock solutions from burettes, and then adding water from another burette to bring the volume to 1 cc. in each case. Next, add 10 cc. of the quinalizarin-sulfuric acid solution. Stopper the vials, cool to 25°C., after which the standards are ready for use. These colors are permanent as long as the vials are kept stoppered to prevent absorption of water.

Determination of available boron in soils

Most of the available boron in soils is undoubtedly water-soluble. It was found that when a known amount of soluble boron in the form of boric acid was added to several soils free of water-soluble boron, and the soils were then dried, the added boron could be completely recovered by adding water, boiling for 5 minutes, and then filtering. The addition of hot water followed by shaking for 30 minutes and then filtering did not result in complete recovery. Complete recovery may be effected by extraction with dilute acid. This procedure, however, raises complications when calcareous soils are encountered, because of the difficulty of regulating the acidity. Furthermore, tests made with acid extractions of calcareous soils indicated that the results thus obtained often do not correlate well with crop indications of the boron status. After numerous tests, refluxing of the soil-water suspension for 5 minutes appeared to be the best procedure. More boron was extracted by refluxing for 5 to 10 minutes than either for shorter or for longer periods. By boiling with a reflux condenser, the volume remains constant, and thus an aliquot is taken more easily later. The details of the analytical procedure finally adopted follow.

It was previously shown (2) that the results thus obtained correlate well with plant response to boron fertilization. De Turk and Olson (3) indicate that water-soluble boron is a fairly reliable measure of the available boron content of soils.

Place a 20-gm. sample of the soil (air-dried and 20-meshed) in a 125-cc. Florence flask (boron-free glass), add 40 cc. of distilled water, and then attach a reflux condenser. Boil for 5 minutes, disconnect the condenser, and filter the suspension with suction on a Büchner funnel or centrifuge until the supernatant liquid is clear. Clarification may be facilitated by adding not more than 0.05 gm. $CaCl_2 \cdot 2H_2O$. Place 20 cc. of the clear extract in a platinum dish and add 5 drops of the K_2CO_3 solution, or place in a porcelain crucible and add 2 cc. of a saturated solution of $Ca(OH)_2$. Evaporate to dryness and ignite gently to destroy nitrates and *all organic matter*. After cooling, add 5 cc. of the approximately 0.36 N H_2SO_4 and triturate thoroughly with a policeman. Filter through 9-cm. paper; by means of a 1-cc. pipette, place exactly 1 cc. of the filtrate in a comparison tube; and by means of the burette and dispensing apparatus illustrated in figure 1, add exactly 10 cc. of the quinalizarin-sulfuric acid solution. Stopper the tube and mix thoroughly by whirling gently. Cool to the desired temperature, and determine the boron content by comparison with a set of standards or by means of the photoelectric colorimeter. The final visual comparison is best made by removing the stoppers momentarily from the tubes and making a vertical observation against a white background, as is usually done in colorimetric comparisons.

Comparison of quinalizarin and curcumin procedures for available boron in soils

The curcumin colorimetric test for boron has been used extensively for many years, and recently Naftel (9), who gives references to the early literature, applied this test in a procedure which he developed for the determination of small amounts of boron in soil and plant extracts. McHargue and Hodgkiss (7) have compared this procedure with other procedures and have concluded that the quinalizarin procedure is to be preferred because of rather wide variations in results obtained at times with curcumin.

For comparison, the curcumin and the quinalizarin procedures were applied to test solutions obtained in the determination of available boron in soils. The curcumin color development procedure was carried out as directed by Naftel (9), and filter number 540 was used in the Evelyn colorimeter. The results obtained are given in table 1. It will be noted that the curcumin procedure, photoelectric comparison, gives practically the same results as the quinalizarin procedure.

TABLE 1

Comparison of results obtained for available boron in soils with the curcumin and quinalizarin colorimetric procedures and precision of visual and photoelectric readings in the quinalizarin procedure

Results in parts per million

	AVAILABLE BORON IN SOILS														
	Curcumin procedure					Quinalizarin procedure									
KIND OF SOIL	Photoelectric comparison					Visual comparison					Photoelectric comparison				
	A	B	C	D	Ave.	A	B	C	D	Ave.	A	B	C	D	Ave.
Plainfield sand..	0.26	0.21	0.26	0.20	0.23	0.225	0.225	0.20	0.225	0.22	0.22	0.22	0.20	0.20	0.21
Miami silt loam.	0.70	0.72	0.63	0.53	0.64	0.60	0.65	0.60	0.60	0.61	0.60	0.67	0.58	0.63	0.64
Clyde silt loam..	1.48	1.22	1.22	1.35	1.32	1.30	1.30	1.30	1.40	1.33	1.50	1.50	1.42	1.28	1.43
Antigo silt loam.	1.30	1.40	1.28	1.30	1.32	1.30	1.20	1.40	1.40	1.33	1.50	1.39	1.32	1.28	1.37

The curcumin procedure has the following advantages: preparation and storage of a strong acid are obviated; difficulties encountered in using strong H_2SO_4, particularly in the photoelectric comparison, are eliminated; and a somewhat wider working range of boron concentrations is possible. The advantages of the quinalizarin procedure are as follows: much less manipulation is needed in filtration, washing, and evaporation, and hence, considerable time and labor are saved; and results are more uniformly accurate because of lessened manipulation.

It will be noted in the data of table 1 that results obtained by visual comparison in the quinalizarin procedure show somewhat less variation than those obtained by either procedure using photoelectric comparison. General experience indicates that after some practice, color comparisons in the quinalizarin procedure can be made visually with an accuracy equalling or surpassing those possible with the photoelectric colorimeter, provided the operator is a good judge of color shades and intensities.

Determination of total boron in soils

The procedure for total boron in soils has not been changed materially from that given in the original report (1).   McHargue and Hodgkiss (7), using essentially this procedure, report satisfactory results in the determination of total boron in soils.

In determining the total boron content of soils by means of the fusion method, it is necessary to use a high proportion of sodium carbonate to soil.   Treatment of the melt obtained with water alone will bring all the boron into solution, but the method is inconvenient because of its slowness and the large quantity of water required.   Addition of sulfuric acid to the water, so that the final reaction of the solution falls within the pH range of 5.5 to 6, hastens the disintegration of the melt and leaves most of the sesquioxides and silica in insoluble form.   Addition of alcohol up to 60 to 70 per cent by volume at this point serves to throw down most of the large amount of sodium sulfate that has been formed.   This leaves all the boron and only a small amount of salts in solution.   After the final evaporation, ignition is necessary because of the small amount of nonvolatile organic matter usually introduced with the alcohol.

Fuse 0.5 gm. of soil with 3 gm. of anhydrous sodium carbonate in a platinum crucible.   Cool and place the crucible in a 250-cc. beaker containing about 50 cc. of distilled water.   Place a cover glass on the beaker and add approximately 4 N sulfuric acid from time to time until the melt has disintegrated and the solution has a reaction in the range of pH 5.5 to 6.0.   Transfer the resulting solution to a 500-cc. volumetric flask.   Wash the beaker and crucible several times with distilled water and add the washings to the flask.   The total volume of solution now should not exceed 150 cc.   Add methyl or ethyl alcohol to the flask until a volume of 500 cc. is reached, and mix the contents thoroughly.   Filter the solution or centrifuge until the supernatant liquid is clear.

Place a 400-cc. aliquot of the clear solution in a 500-cc. beaker (boron-free glass) and add 100 to 150 cc. of distilled water to prevent subsequent precipitation.   Add potassium carbonate until the solution is alkaline, evaporate to a small volume, and transfer to a platinum dish.   Evaporate to dryness and ignite just enough to destroy organic matter.   After cooling, add 4 cc. of approximately 0.36 N sulfuric acid, and triturate thoroughly with a policeman.   Place a 1-cc. aliquot of this solution in a comparison tube, add 10 cc. of the quinalizarin-sulfuric acid solution, stopper the tube, and mix thoroughly by whirling gently. Cool to the desired temperature and then make color readings.

Determination of total boron in plant materials

It was previously shown (1) that it is not necessary to add a base to the plant material to prevent loss of boron during ashing.   McHargue and Hodgkiss (6) say that the use of a base to prevent loss of boron during ashing at a temperature of 450°C. is not necessary.

Place a 0.25- to 0.50-gm. sample of plant material (oven-dried and ground) in a platinum crucible or porcelain evaporating dish and ignite gently to a white or gray ash.   After cooling, add 5 cc. of approximately 0.36 N sulfuric acid and

triturate with a policeman. After filtering, place 1 cc. of the clear filtrate in a comparison tube, add 10 cc. of the quinalizarin-sulfuric acid solution, stopper the tube, and mix thoroughly by whirling gently. Cool to the desired temperature and then make color readings.

PRECAUTIONS

Since many chemicals commonly used contain appreciable amounts of boron, it is essential that all chemicals used in the determination of boron be tested for freedom from this element. Pyrex glass contains about 11 per cent of boric oxide and may cause serious contamination if used in this determination. All liquid reagents should be stored in containers made of boron-free glass. Common soft-glass bottles are usually satisfactory.

Great care must be exercised in measuring the 1-cc. aliquot of the unknown to which are added the reagents for color development, because an error of 1 drop in this measurement may cause an error of from 5 to 10 per cent in the final result through its influence on the final acid concentration in the mixture. In the quinalizarin procedure, contact of the quinalizarin-sulfuric acid solution with moist air should be scrupulously avoided both before and after the reagent is dispensed.

SUMMARY

The quinalizarin color reaction for the determination of boron in various materials is giving satisfactory results and has come into general use. Improvements made in the procedure since the first report on the method include the use of a combination quinalizarin-sulfuric acid solution and special equipment for storing and dispensing this reagent. It has been found that the color reaction is virtually instantaneous, and that the color readings can be made as soon as the test solution containing the color reagent has been cooled to room temperature.

Use of a photoelectric colorimeter for making the color readings has proved to be satisfactory when proper precautions are taken in the selection and care of colorimeter tubes for holding the test solution. Visual readings by means of comparator tubes give equally satisfactory results.

Detailed directions are given for the application of the quinalizarin reaction to the determination of the available boron of soils extracted with boiling water, total boron of soils brought into solution by fusion with sodium carbonate and treatment with weak acid, and the total boron of plant materials brought into solution by ashing and extraction with dilute acid.

The procedure involving use of quinalizarin was compared with a procedure involving use of curcumin for development of color. Both gave equally satisfactory results. The former is less laborious and more expeditious, whereas the latter has the advantage of using reagents that are more easily prepared and handled.

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CHEMICAL AND BIOLOGICAL STUDIES ON AQUEOUS SOLUTIONS OF BORIC ACID AND OF CALCIUM, SODIUM, AND POTASSIUM METABORATES [1]

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Since boric acid is such a weak acid, the borates undergo considerable hydrolysis, and their aqueous solutions are always alkaline. The salts precipitated from aqueous solutions are not normal salts, since boric acid gives up only one hydrogen ion, and they are derived from ortho, meta, and tetraboric acid in a way which cannot be anticipated (5). The condensation of orthoboric acid is brought about by the elimination of water and is favored by a high concentration of hydroxyl ions. If suitable cations are present, they may replace hydrogen atoms of the weakly dissociated metaboric acid, and one of the condensed borates results.

In a soil there may exist an equilibrium mixture of several ionic species, orthoborates which are relatively unstable in aqueous solution, metaborates, and tetraborates. This equilibrium continuously shifts because of fluctuations in moisture content, pH, and quantity and nature of bases. Low soil moisture, high pH, and a high concentration of cations, particularly calcium, all tend to accentuate boron deficiency in plants. The same conditions tend to favor the formation of condensed borates. It is known that in its solid phase the acid radical of calcium metaborate is an endless chain of BO_3 groups, whereas the acid radical of sodium and of potassium metaborate is smaller and of discrete size. Whenever calcium (or magnesium) is the predominating cation, boron absorption by plants is reduced to a greater degree than it is when sodium or potassium is abundant. These relationships suggest that the condensed metaborates may be important as sources of boron to plants and that the different effects of calcium and of sodium or potassium may in some way be related to the chemical behavior of their metaborates, which differ markedly in the solid phases. It was the purpose of this investigation to study the chemical behavior of the metaborates of calcium, sodium, and potassium in aqueous solution with reference to ultimate absorption and utilization of boron by plants.

REVIEW OF LITERATURE

By means of x-ray studies it has been shown that the acid radical in calcium metaborate (CaB_2O_4) is an endless chain of linked BO_3 groups running through the crystal (21). The boron atom is surrounded by three oxygen atoms and occupies the center of an equilateral triangle. Two of these oxygen atoms are shared with other BO_3 groups and in this way endless chains of $(BO_2)_n$ groups are built up. The chains are linked laterally with calcium atoms, each of which is surrounded by eight oxygen atoms. The anion may be as long as the crystal itself. It can be considered to be in a "condensed" state.

In contrast to this type of structure, the acid radical of potassium metaborate $(K_3B_3O_6)$ is of discrete size consisting of three atoms of boron and six of oxygen (23). Each boron atom is linked with three oxygen atoms forming a nearly equilateral triangle. The BO_3 triangles are linked together to form $(B_3O_6)^{-3}$ radicals, and all nine atoms lie in the same plane.

The crystal structure of sodium metaborate $(Na_3B_3O_6)$ is similar to that of potassium metaborate described above (15). Sodium is substituted isomorphically for potassium, making the case somewhat rare from the standpoint of differences between the sodium and potassium atoms. Both can be considered to be in a "condensed" state.

Boric acid contains layers of $B(OH)_3$ molecules held together by hydrogen bonds (22). Each oxygen forms two hydrogen bonds and these are coplanar with the BO_3 groups. Orthoboric acid is condensed by the elimination of water.

Polymerization of the metaborate groups has taken place rather than a reduction of the coördination number of boron from three to two. Condensation is favored by the elimination of water and by a high concentration of hydroxyl ions, since oxygen bridges will be formed and hydrogen atoms eliminated. If calcium ions are abundant they may replace hydrogen ions of the slightly dissociated metaboric acid and form giant radicals of infinite length. If sodium or potassium ions are abundant, they may replace the hydrogen ions and form radicals of discrete size. The conditions that favor the formation of metaborates from boric acid are, therefore, a high concentration of hydroxyl ions, low moisture, and the presence of suitable cations.

In soil media it has been shown that a high concentration of calcium and/or magnesium causes a reduction in the absorption of boron by plants whenever the pH is high (3, 9, 10, 11, 13, 14, 19). On the other hand, boron absorption is interfered with to a lesser extent by the presence of sodium or potassium ions (3, 10, 19). These relationships have been established by greenhouse and field experiments where chemical analyses of plant tissue and observations of deficiency and toxicity symptoms have been used as criteria.

It has been observed repeatedly[3] that low soil moisture tends to cause a reduction in boron absorption by plants (16, 20).

The effect of the four cations upon the absorption of boron by plants in sand cultures is not well understood. Fewer experiments on this phase of the problem have been reported, and the results are not so consistent. Drake, Sieling, and Scarseth (4) observed no effect of varying the calcium concentration on the uptake of boron by corn or tobacco from sand cultures at different pH values. Midgley and Dunklee (9) reported that the minimum lethal dose of boron for flax grown in washed quartz sand was approximately 20 pounds boric acid per acre whether calcium carbonate was present or not. They contrasted this behavior to the beneficial effects of lime in overcoming toxicity of excess boric acid added to soils. Ferguson and Wright (6) found a rather significant difference between equal concentrations of boron from sodium and calcium borates. They grew turnips in sand and found that roots in the sodium borate cultures were healthy, whereas about 50 per cent of those in the calcium borate cultures showed evidence of boron deficiency.

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[3] Lorenz, O. A. 1941 A study of internal breakdown of garden beets with special reference to boron-cation relationships. [Unpublished thesis. Copy on file Cornell University Library, Ithaca, N. Y.]

In evaluating the biological literature with reference to the known facts regarding boric acid and the metaborates of calcium, potassium, and sodium, the following facts become apparent:

Under conditions of high pH and low moisture, the formation of "condensed" borates is favored.

Whenever calcium ions are present in a high concentration in a soil medium, the formation of a condensed metaborate with an endless chain-like structure is favored, and under these conditions boron absorption by plants is greatly reduced.

Whenever sodium or potassium ions are present in high concentrations in a soil medium, the formation of condensed metaborates the molecules of which are of discrete size is favored, and under these conditions boron absorption is interfered with to a lesser degree.

EXPERIMENTAL

Specific conductivity determinations

The purpose of this experiment was to determine the ionic conductance of the boron-containing anion in each of the compounds named above. The general plan was to determine equivalent conductances at infinite dilution and to apply Kohlrausch's law to calculate the ionic conductances of the anions by extrapolation. The ionic conductances of the cations at infinite dilution are known, of course, with considerable accuracy. By using this method of approach it was thought possible to determine whether or not a highly polymerized molecule existed in the calcium metaborate solution.

Since the salts precipitated from aqueous solution are not normal salts, crystals obtained from a fused mass were obtained for preparing the metaborate solutions. Solid calcium metaborate was prepared according to the method used by Zachariasen (21). A mixture of 1 CaO and 1 B_2O_3 was heated in a platinum crucible, and the melt was swirled several times before removal from the furnace. Needle-shaped crystals several millimeters long were formed. Refractive indexes were found to agree with the values of calcium metaborate given by Winchell (18, p. 214).

Two melts were made. Throughout this paper these are referred to as CaB_2O_4, A, and CaB_2O_4, B. Approximately 15 gm. of the crystals were placed in a paraffined bottle containing approximately 500 ml. conductivity water. The solutions were shaken occasionally for 48 hours and allowed to stand for 72 hours. The supernatant liquid was then siphoned off, diluted with 20 to 25 ml. water and analyzed for calcium. The ratio of calcium to boron was according to the formula, CaB_2O_4. Dilutions were made on the basis of calcium determinations until the concentration was either 1/256 or 1/512 molar, depending upon the concentration of the analyzed solution.

The melts for solutions A and B were made January 7. An insufficient volume of solution was prepared, however, and on January 28 the solids with approximately 25 ml. water were transferred to 2-liter paraffined acid bottles. At the same time two additional melts were prepared, and in the manner already described the four new solutions were made exactly 1/256 or 1/512 molar. The new solutions prepared from the old melts are referred to in this paper as CaB_2O_4, A', and CaB_2O_4, B', and those from the new melts as CaB_2O_4, C, and CaB_2O_4. They were stored in tightly stoppered paraffined bottles for later use.

"Magnesium metaborate" crystals were prepared by melting magnesium oxide and boric acid according to the formula MgB_2O_4. Aqueous solutions prepared from the melt did not contain boron and magnesium in this ratio. In view of this behavior and lack of specific information on crystal structure and means of identifying magnesium metaborate when prepared under these conditions, data were not obtained on these solutions.

Crystals of sodium metaborate were prepared by melting sodium carbonate and boric acid in quantities corresponding to $NaBO_2$. Optical properties of this compound were examined and found to agree with those reported by Cole, Scholes, and Ambert (1). Solutions 1/16 molar with respect to sodium were prepared by dissolving the solid directly. The two solutions were referred to as $Na_3B_3O_6$, A, and $Na_3B_3O_6$, B.

Crystals of potassium metaborate were prepared in a manner identical to that described for the corresponding sodium compound. The optical properties were likewise examined, and the solutions are referred to as $K_3B_3O_6$, A, and $K_3B_3O_6$, B.

The sodium tetraborate solution was prepared from c. p. salt recrystallized three times from conductivity water. The solid was dried to expel all water, and a solution 1/16 molar was made by direct weighing.

Specific resistances were determined upon aqueous solutions of these salts at 25°C. ± 0.02° by means of a Jones conductivity bridge.[4] The conductivity water was redistilled from an alkaline permanganate solution and had a specific conductance of not more than 1.6×10^{-6} mho. Water corrections were made to all conductance measurements. Determinations of pH were made on the solutions by means of the glass electrode. Specific conductances were calculated and the conductance due to the various cations was added to that from the hydroxyl ions and the remainder was considered to be due to the boron-containing anion. This value at the most dilute concentration was used as a basis for calculating the ionic conductance of the boron-containing anion. Cumulative errors are naturally expressed in this value.

The results for the sodium metaborate, sodium tetraborate, and potassium metaborate solutions are presented in figure 1 and in table 1. They show that the value obtained for the ionic conductance for the boron-containing anion is essentially the same for all three salts, around 33. This agrees with the literature value of the H_2BO_3 anion[5] and lends support to the belief that in the ionized form, boron exists as $H_2BO_3^-$ in aqueous solutions of sodium tetraborate, sodium and potassium metaborates. Apparently the solutions were stable, for the conductance values did not change with time as indicated by the agreement between determinations made on January 21 and May 28.

[4] Appreciation is expressed to J. G. Kirkwood, department of chemistry, Cornell University, for the use of the Jones conductivity bridge.

[5] Gmelin-Meyer, *Handbuch* (Bor), p. 86, gives 36.8 at 25° for $H_2BO_3^-$ (from H_3BO_3). I. C. T., VI, p. 260, gives 32.8 at 18° for $H_2BO_3^-$.

Landholt Bornstein 5 Auflage II, p. 1089, gives 36.8 for $H_2BO_3^-$ at 25° from $Na_2B_4O_7$ (V = 1024), 39.0 from $Na_2B_2O_4$ (V = 1024).

Jones (Mellor, V, p. 73) gives 31.9 at 25° (V = 4096) from $Na_2B_4O_7$.

Lunden (*Jour. Chim. Phys.* 5: 574, 1907) gives at infinite dilution and at 25°C., 32.7 for $H_2BO_3^-$ from $NH_4H_2BO_3$ and 37.9 for $H_2BO_3^-$ from NaH_2BO_3.

The results for the calcium metaborate solutions are presented in figure 1 and in table 2. The equivalent conductance data show that at the higher concentrations, aqueous solutions of calcium metaborate are relatively poorer conductors than the sodium or potassium metaborates. Greater ionic strength of the calcium metaborate solutions or greater polymerization at higher concentration may account for this behavior. In all cases the value for the ionic conductance of the boron-carrying anion is lower than the literature value of $H_2BO_3^-$ or the value obtained above from sodium and potassium borates. The low values for solution A obtained on January 21 cannot be explained.

Instability of the system is shown by the lowering of conductance with time, and a lowering of pH toward neutrality. This may represent a gradual "aging"
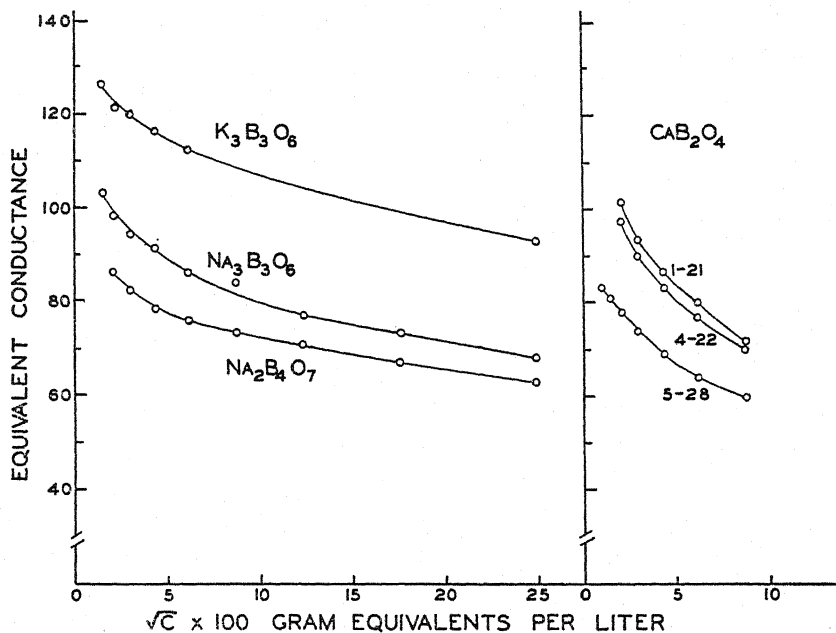
FIG. 1. EQUIVALENT CONDUCTANCE VALUES FOR FOUR BORATES

process in which the equilibrium between the several possible ionic species is changing. Polymerization is suggested by the poor and changing conductance.

The possibility of an effect from carbon dioxide contamination is not removed, however, for the solutions were not kept in a CO_2-free atmosphere. The water used in their preparation was CO_2-free at the beginning, and the bottles were kept tightly stoppered. They were opened not more than five or six times during the entire storage period. In pure carbonate systems at 16°C., Johnston and Williamson (7) have shown that the minimum concentration of calcium ions occurs at a carbon dioxide concentration approximately $\frac{1}{100}$ that of the air and produces a concentration of 1.46×10^{-4} moles per liter, which is approximately $\frac{1}{13}$ that of solutions A′ and C (1/512 molar). Solutions B and D are twice this concentration and if poorer conductance were due to precipitated carbonate, it would seem a greater "aging" effect should occur in the more concentrated solu-

tion where the possibility of exceeding the solubility product is greater. As shown in table 2, however, this was not found to be true. During the storage period the conductance of solutions B′ and D decreased no more than those of solutions A′ and C, which were only half as concentrated.

TABLE 1

Conductance data on two sodium metaborate, one sodium tetraborate, and two potassium metaborate solutions at 25°C.

LITERS CONTAINING 1 GM. EQUIVALENT	SPECIFIC CONDUCTANCE, MHOS × 10⁵		pH A AND B	OH⁻ NORMALITY × 10⁵	SPECIFIC CONDUCTANCE, MHOS × 10⁵ FROM				IONIC CONDUCTANCE OF BORON-CONTAINING ANION	
	Soln. A	Soln. B			Na⁺ (or K⁺)	OH⁻	Boron anion		A	B
							A	B		
$Na_3B_3O_6$	1/21	1/21	4/11							
16	430.53									
32	230.94									
64	122.70									
128	65.75									
256	33.83	33.90	9.95	8.91						
512	18.12	17.70	9.87	7.41						
1024	9.18	9.25	9.73	5.37						
2048	4.73	4.87	9.58	3.80	2.43	0.74	1.56	1.70	31.9	34.8
4096	2.45	2.59	9.37	2.35	1.22	0.46	0.77	0.91	31.6	37.3
		5/28	6/11							
16		430.37	10.47							
128		64.37	10.22							
1024		9.25	9.78							
$Na_2B_4O_7$	1/21		4/11							
1024	8.06		8.80	0.63	2.43	0.12	1.48		30.3	
2048	4.24		8.60	0.40	1.22	0.08	0.82		33.6	
$K_3B_3O_6$	1/21	1/21	4/11							
16	583.00	586.20								
256	43.67	44.22	10.06							
512	22.60	22.92	9.93							
1024	11.62	11.82	9.78							
2048	5.95	5.90	9.61	4.07	3.57	0.80	1.58	1.53	32.4	31.3
4096	3.08	3.08	9.39	2.45	1.79	0.48	0.81	0.81	33.2	33.2
	5/28	5/28	6/11							
16		586.11	10.57							
128		84.71	10.23							
1024		11.79	9.69							

Electrodialysis of aqueous solutions of calcium, sodium, and potassium metaborates

The purpose of this experiment was to determine whether or not there was any difference in the behavior of the metaborates with respect to the quantity of boron caused to pass through a membrane under the influence of a given quantity of electricity. Aqueous solutions of sodium, potassium, and calcium metaborates in 100-ml. quantities, each containing 100 p.p.m. B, were placed successively in

the middle compartment of a three-compartment Bradfield cell. A current of approximately 0.02 ampere was caused to pass for approximately 30 minutes, the exact quantity of electrical current being measured by means of a silver coulometer in the circuit. Immediately after the current was turned off, the solution

TABLE 2

Conductance data on four calcium metaborate solutions at 25°C.

LITERS CONTAINING 1 GM. EQUIVALENT CaB_2O_4	SPECIFIC CONDUCTANCE, MHOS × 10⁵ Data obtained 1/21				pH 4/11	OH⁻ NORMALITY × 10⁵	SPECIFIC CONDUCTANCE, MHOS × 10⁵ FROM						IONIC CONDUCTANCE OF BORON-CONTAINING ANION			
							$\frac{1}{2}Ca^{++}$	OH⁻	Boron anion				A'	B'	C	D
	A	B							A'	B'	C	D				
128	13.20	56.30														
256	6.76	31.50														
512	3.65	17.00														
1024	1.87	9.18														
2048	1.01	4.98			9.56	3.36	2.98	0.71		1.29				26.4		
	Data obtained 4/22															
	A'	B'	C	D												
128		54.95		55.22	10.17											
256	30.67	30.34	29.41	30.44	10.11											
512	16.53	16.49	15.98	16.54	9.93											
1024	8.90	8.93	8.60	8.84	9.75											
2048	4.83	4.85	4.64	4.81	9.56	3.63	2.98	0.71	1.14	1.16	0.95	1.12	23.2	23.8	19.5	22.9
	Data obtained 5/28				6/11											
	A'	B'	C	D												
128		48.93		48.33	9.69											
256	21.73	26.86	25.54	26.56	9.57*											
512	11.70	14.56	13.71	14.35	9.55											
1024	6.20	7.79	7.36	7.67	9.46											
2048	3.27	4.10	3.90	4.08	9.34	2.19	2.98	0.43		0.69	0.49	0.67		14.1	10.0	13.7
4096	1.68	2.12	2.01	2.11	9.20	1.59	1.49	0.31		0.32	0.21	0.31		13.1	8.6	12.7
8192	0.84	1.10	1.06	1.09	8.85	0.71	0.74	0.12		0.24	0.20	0.23		19.7	16.4	18.8

* The pH values for solution A' for this dilution and for the five following were: 9.45, 9.38, 9.27, 9.26, 9.00, and 8.84.

remaining in the middle compartment was siphoned off. Anolyte and catholyte compartments were analyzed for boron by the turmeric method (12).

The results are presented in table 3. They show that boron migrates as part of an anion at somewhat different rates for the three salts but that its behavior is not markedly different. There is no evidence of reversed electrolysis, qualitative tests for sodium, potassium, and calcium showing that these ions were present in their respective catholytes only.

Solubility of calcium metaborate

The purpose of this experiment was to study the effect of time and the amount of solid phase present on the solubility of calcium metaborate.

A concentration of 650 p.p.m. B was obtained by allowing 30 to 40 gm. solid calcium metaborate to remain in contact with approximately 100 ml. water for 6 months. On the other hand, difficulty was encountered on several occasions when an attempt was made to dissolve, over a period of 30 days, a quantity of calcium metaborate calculated to give about one-tenth this concentration. This suggested that the solubility of calcium metaborate was influenced by the amount of solid phase present and by the time of contact.

TABLE 3

Boron migration through a cellophane membrane during the electrodialysis of aqueous solutions of sodium, potassium, and calcium metaborates containing 100 p.p.m. B

SALT	CURRENT PASSED	TOTAL B FOUND		B PER COULOMB FOUND	
		Anolyte	Catholyte	Anolyte	Catholyte
	coulombs	γ	γ	γ	γ
$Na_2B_2O_6$	37.4	70	2	1.8	0.1
	39.9	86	Tr	2.2	Tr
	32.8	88	Tr	2.7	Tr
Average				2.2	
$K_2B_2O_6$	33.5	115	7	3.4	0.2
	31.4	122	6	3.9	0.2
	36.2	130	3	3.6	0.1
	38.6	135	2	3.5	0.1
Average				3.6	
CaB_2O_4	36.8	83	6	2.3	0.2
	36.6	100	Tr	2.7	Tr
	35.2	99	Tr	2.7	Tr
Average				2.6	

To a series of 500-ml. glass-stoppered bottles, finely ground calcium metaborate prepared from fusion as described earlier in this report was added in amounts to give solutions containing approximately 2, 1, $\frac{1}{2}$, and $\frac{1}{4}$ gm. per liter. The exact quantities were selected so concentrations in terms of liters containing 1 gram-molecular weight of CaB_2O_4 were: 64, 128, 256, and 512.

The bottles were shaken at intervals for several days, an attempt being made to keep the shaking treatments comparable but not necessarily to dissolve the maximum possible under these conditions. The solutions were allowed to settle for at least 12 hours before 2-ml. aliquots were taken for analysis at the end of 4 days and 9 days. The 2-ml. aliquots were analyzed for boron by the turmeric method. For the final analyses after 41 days, larger aliquots were taken and analyzed by the mannitol titration method (17). The results presented in figure 2 show the amount of boron in solution at the end of the three time intervals in

relation to the maximum concentrations which could be derived from the various quantities of solid phase.

The results show that the amount of boron in the supernatant liquid depends upon the amount of excess solid phase and the time of contact. The dissolving of calcium metaborate is not in accordance with true solubility behavior. Some very slow reaction is apparently taking place in the system, and this may be similar in nature to that known to occur in calcium metaphosphate systems (8). Hydrolysis is likely a factor and there is no insurance that true solubility alone was being measured. The "aging" effect as shown by conductivity measurements may be related to a similar phenomenon.
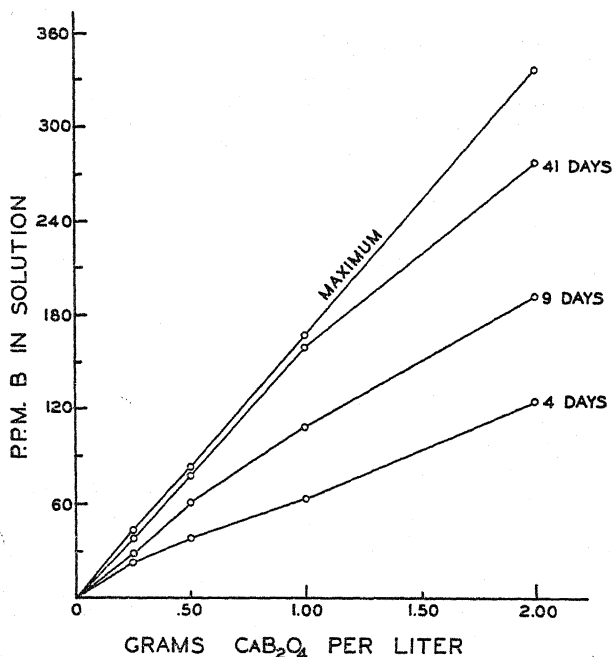
FIG. 2. SOLUBILITY OF CALCIUM METABORATE AS A FUNCTION OF TIME AND THE AMOUNT OF SOLID PHASE ADDED

Whatever the mechanism is, it seems probable that the slow dissolving rate of calcium metaborate is responsible for reductions in boron absorption by a plant whenever a soil medium undergoes pronounced fluctuations in moisture. Since the supply of boron to a plant must be continuous, it is conceivable that boron deficiency could be induced even if the water-soluble boron content of the soil were relatively high.

Absorption of boron by plants in water cultures

The purpose of this experiment was to determine whether or not boron was absorbed by the sunflower plant at equal rates from equivalent concentrations of aqueous solutions of calcium, sodium, and potassium metaborates and boric

acid. Sunflower seeds of a giant Russian variety were germinated in washed quartz sand. When they were 5 days old they were transplanted to bottles filled with 500 ml. complete nutrient solution which was 0.0180 molar with respect to KH_2PO_4, 0.0052 molar to $Ca(NO_3)_2$, and 0.0150 molar to $MgSO_4$. Five milliliters of a stock solution containing 0.20 gm. $MnCl_2 \cdot 4H_2O$, 0.10 gm. $ZnCl_2$, and 0.02 gm. $CuCl_2 \cdot 2H_2O$ was added per liter of nutrient solution. Boric acid was added to the nutrient solution to supply 0.01 p.p.m. B. One milliliter of a stock solution containing 5 gm. ferric tartrate per liter was added to each culture separately every 2 or 3 days.

The plants were allowed to grow in this solution for 9 days, or until the first indications of boron deficiency became apparent. At this time, the fourteenth day, differential boron treatments were begun. Solutions of boric acid and of sodium, potassium, and calcium metaborates were made up to supply the following concentrations of boron from each source: 0.01, 0.02, 0.10, 1.0, and 5.0 p.p.m. B. The sodium and potassium metaborate solutions were prepared by diluting the stronger solutions which had been made up from the melts as described. The calcium metaborate solution designated as 4 was prepared by making dilutions on the stronger solution made from melt "D". The "calcium metaborate" solution designated as 5 was prepared by adding a boric acid solution to $Ca(OH)_2$ to give calcium and boron, according to the formula, CaB_2O_4. A set of four plants from one of the uniformly treated cultures was transferred to each of the different boron solutions after the roots were carefully washed off with distilled water. The exact time of each transfer was recorded. The cultures were run in duplicate.

During the 4-hour absorption period (June 8) the plants were placed outside the greenhouse, the first transfers being made at 2:00 p.m. A strong breeze was blowing, the temperature fluctuated between 70 and 80°F., and the relative humidity was around 50 per cent. Light intensity was 9,000 foot-candles at 2:00 p.m., and 7,000 at 6:30 p.m. At the end of the 4-hour absorption period the two older pairs of leaves were cut from one plant in each culture. They were approximately 2 to $2\frac{1}{2}$ inches long and were taken from plants the newest leaves of which were 5 ± .5 inches above the top of the container. The leaves were dried at 70°C. for 48 hours, and boron determinations were made by the turmeric method (12). Samplings were made after 18 hours and 42 hours in a similar manner. From the fourth through the eighteenth hour the plants were placed in the darkened headhouse with a temperature of 70 to 80°F., and a relative humidity which fluctuated between 50 and 70 per cent. From the eighteenth through the forty-second hour they were in the greenhouse, where the temperature varied between 65 and 85°F. and the relative humidity from 30 to 60 per cent. Light intensity reached a maximum of 7,000 foot-candles during this period.

The results of this investigation are presented in figure 3. A measurable quantity of boron was absorbed from all cultures during the 4-hour absorption period. The concentration of boron in the plants growing in the 0.01 p.p.m. B cultures nearly doubled, whereas that in the plants growing in the 5.0 p.p.m. B

cultures increased approximately fivefold. The low-concentration plants did not gain much more boron in the following 14 hours, but the plants in the 5.0 p.p.m. B cultures had increased their original boron contents to around thirteenfold or fourteenfold. These data show that the amount of boron absorbed by the sunflower from aqueous solutions of boric acid and of several metaborates is directly related to the concentration of boron in the medium and to the time of absorption, not to the salt which furnishes it.
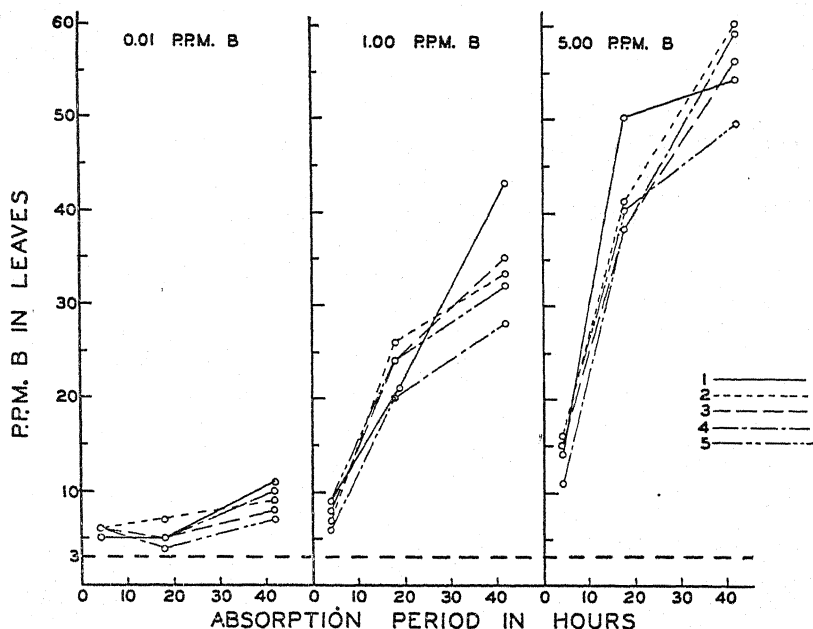
FIG. 3. ABSORPTION OF BORON, BY SUNFLOWERS, FROM AQUEOUS SOLUTIONS OF SEVERAL COMPOUNDS

The concentration of boron in comparable leaves prior to treatment was 3 p.p.m. B. 1. boric acid; 2. sodium metaborate; 3. potassium metaborate; 4. calcium metaborate (from fused salt); 5. "calcium metaborate" (from calcium hydroxide and boric acid).

Absorption of boron by plants in sand cultures

The purpose of this experiment was to determine whether or not aqueous solutions of the metaborates of sodium, potassium, and calcium were as good a source of boron for sunflowers in a sand medium as was boric acid. The general plan was to grow sunflowers in sand to which boron from each of the sources was added in insufficient quantities for normal growth (0.25 p.p.m. B, weight basis) and in excess quantities (2.05 p.p.m. B, weight basis). The criterion of boron absorption was the appearance of boron deficiency or toxicity symptoms. The age of the culture at the time each of four plants first showed the symptoms was recorded and used as a measure of the degree of injury from either condition.

Details of procedure and results of the investigation are presented elsewhere (2). It was found that regardless of the source of boron, the deficiency symptoms

appeared when the cultures were about 25 days old and the toxicity symptoms at about the twentieth day. The effects of high cation concentrations upon boron absorption under these conditions were not determined.

SUMMARY

A study of aqueous solutions of calcium, sodium, and potassium metaborates and of sodium tetraborate was made in pure chemical systems and in relation to absorption of boron by plants.

Specific conductance determinations were made on increasingly dilute solutions of these compounds, and from these data the ionic conductance of the boron-carrying anion was calculated. For the sodium and potassium salts this value was around 33, which corresponds with the literature value for the H_2BO_3 anion. For the calcium salt this value was lower and the system was unstable as indicated by a gradual lowering of the conductance values and of pH towards neutrality. A fundamental difference between the behavior of aqueous solutions of calcium metaborate and sodium or potassium metaborates is shown by these measurements.

Aqueous solutions of these metaborates were electrodialyzed through cellophane membranes, and it was found that boron migrates as part of an anion at somewhat different rates for the three salts but that its behavior is not markedly different. There was no evidence of reversed electrolysis.

Studies on the solubility of calcium metaborate showed that the amount of boron in the supernatant liquid depends upon the amount of excess solid phase present and the time of contact. Concentrations of 650 p.p.m. B were obtainable when an excess of 30 to 40 gm. was in contact with approximately 100 ml. water for 6 months. On the other hand, with periodic shaking, not all of the 0.2455 gm. CaB_2O_4 per liter (42.3 p.p.m. B) dissolved in 41 days. The dissolving of calcium metaborate is not in accordance with true solubility behavior. Some very slow reaction is apparently taking place within the system, a concept supported by the slow "aging" effect as discovered by conductivity measurements. Hydrolysis of a nature similar to that known to occur in calcium metaphosphate systems may be a factor of importance in aqueous solutions of calcium metaborate. The slow dissolving rate of calcium metaborate undoubtedly has considerable practical significance.

In a water culture experiment with the sunflower, a study of the absorption of boron from aqueous solutions of boric acid and of calcium, sodium, and potassium metaborates was made. The results showed that the amount of boron absorbed by the plant was related to the concentration of boron in the substrate and to the time of absorption and not to the salt which furnished it.

In sand cultures to which 0.25 p.p.m. B was added from solutions of boric acid and of calcium, sodium, and potassium metaborates, it was found that boron-deficiency symptoms appeared when the cultures were about 25 days old, regardless of the source of boron. To a comparable series, 2.05 p.p.m. B was added, and it was found that toxicity symptoms appeared after approximately 20 days, regardless of the source of boron.

The absorption of boron by plants does not depend upon its source so long as the water-soluble concentration is the same.

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HISTOLOGIC-PATHOLOGIC EFFECTS OF BORON DEFICIENCY

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It has been recorded by many investigators with numerous species of plants that boron deficiency produces pathologic symptoms most pronouncedly in those regions where meristematic growth is active. The purpose of this communication is to detail some of the histologic changes which occur as a result of boron deficiency. The illustrations are drawn from work which was carried out with the garden beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* var. *capitata* L.).

Plants used in this study were from sand cultures in which boron-starved and boron-supplied plants were produced and fixed at various intervals. The results obtained from such plants were compared with normal and boron-deficient plants produced under field culture.

The beet is a plant in which a relatively unusual condition exists in the development of meristem, particularly in the root. Most of the enlarged storage organ is derived from a series of adventitious cambiums which are formed successively within the expanding periderm and without the primary and secondary xylem. A partly grown root consists primarily of a series of rings, each consisting of an active cambium the cells of which normally differentiate into xylem cells on the side toward the center of the root and into phloem cells on the side toward the periphery. These rings, called "tertiary rings," are youngest and normally most active near the periphery, slowing down in activity and increasing in amount of differentiated, and thus relatively fixed, tissue progressively toward the center.

The internal breakdown associated with boron deficiency commonly follows the configuration of the tertiary rings. It may be most pronounced near the center, near the periphery, or roughly between the center and the periphery of the root. This is particularly true in field-grown beets where transient environal conditions influence the rate of growth, and presumably the availability of boron to the meristematic regions varies from week to week. The greatest breakdown occurs in the rings most active at the time boron deficiency becomes most acute.

When boron starvation is brought about gradually in sand cultures, the progressive effects on the histology of the plant can be studied in successive tertiary rings. Long before any macroscopic evidence appears, various profound microscopic changes occur.

One of the effects of boron starvation is that of increased cell division in the cambium accompanied by decreased cell differentiation. Pronounced necrosis is seldom the first reaction. On the contrary, increased cell metabolism is the most common reaction to boron starvation. Normal cell-wall differentiation, however, is upset, and various manifestations characteristic of pathological cell division due to various causes follow. When cells do not differentiate normally, in this case into various xylem and phloem elements, there is a tendency for cell

division to continue abnormally and for some cells to grow abnormally large. Cell walls remain thin, and parenchyma tissue increases at the expense of conductive tissue. Thus, one of the first effects of boron deficiency is to increase cell number and size and to decrease cell differentiation in the meristematic regions. The same effect may be seen in the vascular system of beet leaf petioles and later in the seed stem. The reduction in the normal development of conductive tissue leads indirectly to many of the characteristic symptoms such as leaf distortion, increased anthocyanin in leaves, stunting of growth, and reduced yield.

It is entirely possible for these disturbances to occur during a temporary shortage of available boron without producing any distinctive symptoms, if the plant recovers in time. Even though macroscopic symptoms do not appear, growth may have been slowed down, however, and yield reduced. This may account for various reports of increased yield following boron applications when boron deficiency symptoms failed to appear.[1] It also accounts for the common observation that boron-deficiency symptoms appear more slowly and less pronouncedly in slow-growing, poorly fertilized beets than in well-fertilized, actively growing ones.

When beets showing definite internal brown spot are examined, it is found that the greatest breakdown occurs in those tertiary rings in which early differentiation of cambium cells into phloem and xylem is under way, and that correspondingly less occurs in older rings where differentiation has proceeded longer.[2] In other words, those meristematic regions that are most active and presumably need most boron are first to exhaust their diminishing available supply and likewise are the first to show acute symptoms.

Other effects of boron deficiency in garden beet take on various forms. Discoloration appears first in a relatively few cells the protoplasts of which turn dark brown as they degenerate. Cell walls can be seen to turn dark in color and cells to collapse. Thus while cell differentiation is interrupted, groups of parenchyma cells die and necrosis sets in. This again appears in the most active rings, and microscopic dark patches appear. Around such patches living cells become more active, producing a sort of wound cambium and temporarily walling off the dead region. If boron starvation continues, these patches become larger and more numerous, often involving groups of differentiated phloem or xylem. Eventually such areas become macroscopic, and typical internal black spot appears.

In the last stages the histologic picture shows, then, first, tremendous increase in meristematic activity, evidenced by increase in cell number and cell size; second, decrease in differentiation, evidenced by reduction in xylem and phloem conductive tissue; and third, necrotic development in the abnormally stimulated parenchyma tissue followed by secondary wound reaction around necrotic patches and eventually merging of necrotic areas to make visible lesions. The important

[1] Walker, J. C., Jolivette, J. P., and McLean, J. G. 1943 Boron deficiency in garden and sugar beet. *Jour. Agr. Res.* 66: 97-123.

[2] Jolivette, J. P., and Walker, J. C. 1943 Effect of boron deficiency on the histology of garden beet and cabbage. *Jour. Agr. Res.* 66: 167-182.

fact, however, is that the pathologic symptoms in the form of necrosis are but the end result of a series of histologic changes which result from abnormal cell physiology and differentiation.

The cabbage, representative of most herbaceous dicotyledonous plants, has a single cambium which continually lays down secondary xylem and phloem during the growing period in stem and root. In young plants the cambium is again that region most profoundly influenced by boron starvation.[2,3] The normal cambium is replaced by a zone of tissue much wider than the normal cambium and containing a much larger number of cells, thin-walled, and in many cases variously oversized and misshapen. Correspondingly xylem and phloem differentiation is reduced in both stem and root. Patches of necrotic areas appear, but normally they are not so conspicuous in young cabbage stems and roots as in beet roots. After midseason, when the storage of reserve foods in the leafy head occurs, the pith of the stem grows rapidly, eventually forming the relatively thick "core" of the head. The growth of this stem pith involves rapid cell division and cell growth. This activity coincides commonly with that part of the growing season when boron deficiency becomes most acute in the field, and it is the "core" of cabbage which shows the most pronounced boron-deficiency symptoms. Little or no abnormality in the roots has been found.

In the pith, cell division and cell enlargement normally proceed without further differentiation, the cells remaining thin-walled repositories for storage materials. It is possible to observe here, then, the effect of boron starvation on a metabolically actively dividing storage tissue in which cell differentiation, such as to xylem and phloem in the beet root, is not normal. The chief first effect is the development of various-sized necrotic patches. In general, such patches consist of thick-walled, often dark colored, and sometimes crushed cells in the center; around these is a zone of abnormally large cells in which abnormal cell-wall thickening is under way; next there is a zone of rapidly dividing thin-walled cells and, intermingled with or next to these in an adjacent zone, cells with scalariform, reticulate, pitted walls quite unnatural to normal healthy pith. Such patches often coalesce to form a distinct black degenerate central lesion in the core of the head.

To summarize, the first pathologic effects of boron deficiency are physiological and tend to speed up cell division and growth. Cell-wall formation and cell differentiation are concurrently interrupted. Necrosis follows, and the by-products released from dead or dying cells may also become factors influencing growth of surrounding cells. In vascular cambium, as in beet root, the result is a deficiency in conducting elements, which leads to secondary effects on plant growth. In storage tissue, as in cabbage pith, necrosis is accompanied by hyperplasia and hypertrophy as well as abnormal differentiation into various types of thick-walled cells.

A common observation in garden beets grown for canning in Wisconsin is the sudden appearance of internal black spot shortly before harvest. This often

[3] Walker, J. C., McLean, J. G., and Jolivette, J. P. 1941 The boron deficiency disease in cabbage. *Jour. Agr. Res.* 62: 573–587.

occurs even though large applications of borax were applied before the crop was planted. Such was the case in the Green Bay area in 1942 after 50-pound applications of borax. It was the case in the Racine-Kenosha area in 1943 where as high as 25 per cent of the beet roots were affected with internal black spot although 75 pounds of borax was broadcast with the fertilizer and thoroughly worked into the soil before planting. The fertilizer application in the latter case was liberal (1500 pounds of 3–12–12 per acre) and growth was particularly rapid until the roots were about half grown. A period of about four weeks with scarcely any rainfall followed, and growth naturally slowed down. Toward the end of this period and about two weeks before harvest, internal black spot appeared. It was found chiefly in the outer tertiary rings.

It is not the purpose of this paper to attempt to explain why this liberal application of borax was inadequate. The picture is a relatively common one. It is clear, however, that the meristematic regions in the outer tertiary rings keep up their activity at a higher rate and for a longer time than adequate boron can be supplied to them. In red beet the signal of boron starvation registers quickly and discernibly in the form of internal black spot, since there are so many cambiums in activity that some one or two are sure to suffer acute shortage. Other species of plants probably are affected physiologically and their normal growth is retarded by disturbed activity of their meristematic regions, but the deficiency symptoms are slower to develop and in fact may not appear at all macroscopically.

The histologic study emphasizes that boron deficiency may retard growth of plants without producing macroscopic symptoms. The differentiation of conducting tissue is first affected and this in turn must affect mineral absorption and distribution and the efficient use of soil nutrients.

BORON NUTRITION OF THE GRAPE

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Although Agulhon's (1) experiments demonstrated, as early as 1910, the usefulness of boron in the nutrition of certain higher plants, practical interest in boron as a beneficial fertilizer element did not develop until after the recognition, 20 years later, by Brandenburg (3) of a boron deficiency of the beet under field conditions. Atwater (2) has aptly pointed out that the symptoms of abnormality in many plants which we now know are correctable by the addition of boron were recognized and described years before the role of boron in plant nutrition was suspected. "Black-heart" of beets, "internal cork" of apples, and "cracked stem" of celery are examples.

Maier's (5) water culture experiments with grape seedlings had served to include the grape in the list of plants for which boron has been shown to be an essential nutrient, but no instance of deficiency under vineyard conditions had been recognized. It is quite probable that growth and fruiting abnormalities in the grape caused by boron deficiency had occurred but either were unrecognized or attributed to other factors. The instance of boron deficiency in the grape reported in 1941 (9) may be such a case.

The present paper is concerned with observations over a period of 5 years on the effect of boron on growth and fruiting of the grape and on the boron content of the vines, in a vineyard which had shown symptoms of boron deficiency.

MATERIALS AND METHODS

The vineyard

The experimental vineyard is in the Sandhill area near Columbia, South Caroline, on a deep phase of Norfolk sand. This soil is very low in organic matter and natural fertility, giving crop response to application of many of the plant-food elements. The vineyard had received annual applications of a "complete" (N-P-K) fertilizer at the rate of 600 to 800 pounds per acre. In addition, 1500 pounds per acre of dolomitic limestone was applied in 1932 and 1000 pounds per acre of basic slag in 1938. The pH of the surface soil was about 5.8 in 1939.

The vineyard is a test block of 50 varieties, which were 5 to 9 years old in 1939, originally planted in groups of five or ten vines of a variety. In June, 1939, two or three vines of each of ten varieties were treated with borax at the rate of 10 pounds per acre applied to the soil in bands on both sides of the vine. In May, 1940, half of the vines of the remaining varieties in the vineyard were treated similarly.

Analytical methods

Boron determinations were made by the quinalizarin method, following the procedure of Maunzell (6) and using a Coleman spectrophotometer in obtaining colorimetric readings.

RESULTS

Boron-deficiency symptoms and remedial effect of borax

Foliar symptoms that suggested the possibility of boron deficiency were noted in June, 1939. These symptoms consisted of a well-developed pattern with chlorotic areas toward the leaf margin and between the leaf veins. Even in severe cases, the chlorotic areas remained intact with little or no breaking down of tissue or burning of the leaf margins. The surface of affected leaves was abnormally roughened with raised areas between the veins, resulting in a cupping of the leaf toward the under side. Premature defoliation did not occur. The new leaves produced after treatment with borax showed no evidence of abnormality, although the older leaves remained chlorotic. However, the late growth on untreated vines showed little evidence of abnormal symptoms.

Shortly after growth started in early May, 1940, it was noticed that the shoots on a number of vines that had not been treated with borax in 1939 were developing abnormally with pronounced stunting of growth and a tendency toward the development of several lateral shoots from a single node, especially from the nodes most distant from the trunk of the vine. The internodes were very short and the leaves small and in many instances misshapen. Flower clusters were developed but were twisted, malformed, and failed to set fruit. Untreated vines of other varieties showed less extreme symptoms, involving only slight chlorosis or cupping of the younger leaves, similar to those observed the previous year. The treated vines of the ten varieties that had received the application of borax in 1939 showed no indications of the symptoms and were developing normally in every respect. By the middle of July, 1940, the new growth, which in the grape consists in the extension of existing shoots, on those vines receiving borax treatment May 22 of that season, was normal in appearance. Again, as in 1939, it was noted that the later growth on the untreated vines was not so badly affected as the early season growth.

In the following season of 1941, the development of symptoms was comparable to that observed previously, with the exception that possibly certain varieties were affected less severely. Of the 100 or more vines of many varieties showing definite symptoms of boron deficiency before receiving borax treatment in May, 1940, only two individual vines failed to show complete recovery and normal growth in 1941.

In 1942 and 1943 boron-deficiency symptoms again appeared on untreated vines but in less extreme form than in the two previous seasons. In general, differences between treated and untreated vines were less pronounced than formerly. Several factors may account for this: first, the effect of the borax treatments in 1939 and 1940 may have been largely dissipated; second, overcropping

of boron-treated vines in 1942 accompanied by very dry weather in late summer and autumn weakened these vines; and third, crop failure on the untreated vines for the several years previously may have resulted in a more favorable plant-soil-boron relationship for these vines.

Varietal differences under boron-deficiency conditions

Great differences in varietal response to the deficiency existed. Ontario, Carman, Armalaga, Lomanto, Seneca, and Geneva are representative of varieties that were severely affected, showing extreme foliar symptoms, stunting of growth in spring, and virtually complete crop failure. Catawba, Bailey, Lenoir, Concord, Extra, Herbert, and Niagara were among those moderately affected, showing some foliar symptoms especially at the time of blossoming and more or less effect on yield. Champion, Portland, Fredonia, R. W. Munson, and Isabella showed few or no foliar symptoms, although fruit production may have been affected in some instances. It may be pointed out that extreme differences in yield and vigor of the many varieties were existent from time of planting in the experimental vineyard. The severity of symptoms, however, was apparently not correlated with inherent vigor of the variety. Thus, although the weak-growing Delaware, Headlight, and Ontario were severely affected, the equally weak Portland and Moore's Early showed little evidence of deficiency. Similarly, the rank-growing Carman, Bailey, and Extra showed symptoms of deficiency, whereas the vigorous vines of R. W. Munson, Cloeta, and Champion remained normal.

Yield response to borax

The effect of the borax application upon fruit production has been even more striking than its effect on vegetative growth. The 1941 yields of 33 varieties receiving borax treatment in 1940 compared with yields of untreated vines are given in table 1. Of these varieties, 28 produced more fruit from the borax-treated vines. In 18 varieties the treated vines yielded more than double the untreated vines, with a number of the untreated vines being virtually barren. The responses in yield were not necessarily in the same order as the vegetative responses. On several varieties boron greatly increased yield even though foliar symptoms were not pronounced on the untreated vines. This is illustrated by the performance of Lenoir, a very vigorously growing variety under Sandhill conditions. Foliar symptoms of the deficiency in these vines were never acute, seldom being more than slight chlorosis and cupping of the leaves. Yet untreated vines produced scarcely any fruit, while the yields of borax-treated vines were extremely heavy.

Another instance of the pronounced effect of boron upon fruitfulness was illustrated in the yield records of the reflex-stamen or self-sterile varieties. All five of the varieties of this type showed greatly increased yields from borax treatment. Fruit clusters of borax-treated vines of Herbert and Last Rose were well filled and equal in appearance to those of self-fertile varieties. Treated and untreated vines were adjacent in the row and should have been equally favored

in cross-pollination.   Treatment did not affect time of blossoming.   The stamens
of both treated and untreated vines were reflexed in the normal manner for

TABLE 1

Effect of applications of borax in 1940 upon yields of grape varieties in 1941

VARIETY	TREATED WITH BORAX		UNTREATED	
	Number of vines	Yield per vine	Number of vines	Yield per vine
		lbs.		*lbs.*
America	1	1.0	2	1.1
Armalaga	5	7.4	5	0.4
Bailey	2	22.8	3	0.9
Barry*	2	5.6	3	0.2
Brighton*	1	4.7	1	0.2
Brocton	2	0.8	1	0.1
Caco	1	5.4	3	3.6
Carman	3	13.0	3	3.2
Catawba	4	3.2	3	0.6
Champagne	2	9.3	1	10.9
Champion	2	12.1	3	12.6
Cloeta	2	3.8	3	3.0
Delaware	6	3.4	3	2.0
Diamond	2	3.8	3	0.1
Edna	2	5.2	3	9.8
Extra	3	18.2	2	7.5
Fern	2	10.9	2	8.0
Fredonia	2	9.9	2	5.0
Hanover	2	6.2	1	0.8
Herbert*	4	8.9	3	0.1
Isabella	2	11.5	4	5.2
Last Rose*	1	14.5	1	0.0
Lenoir	5	22.5	5	2.2
Lucile	2	3.9	4	1.3
Lukfata*	9	11.3	9	6.5
Lutie	2	2.4	4	1.6
R. W. Munson	2	8.3	3	7.7
Niagara	2	4.1	4	1.5
Ontario	2	5.5	2	0.2
Portland	4	3.8	5	2.7
President	1	3.4	1	0.4
Vergennes	2	4.4	1	4.9
Worden	1	1.2	1	1.0
Average for all varieties		7.6		3.2

* Reflex-stamen varieties.

varieties of this type, and a number of clusters bagged in 1942 failed completely
to set fruit either on treated or untreated vines.

Boron content of vines

Leaf samples from boron-treated and untreated vines of 14 varieties were
analyzed for boron May 1, May 29, August 9, and September 10, 1941, and on

TABLE 2

Boron content of leaf samples from borax-treated and untreated vines

In p.p.m. of dry material

VARIETY	BORON TREATMENT*	MAY 1, 1941	MAY 29, 1941	AUG. 9, 1941	SEPT. 10, 1941	JUNE 17, 1942	SEPT. 11, 1942	VARIETY MEANS BY TREATMENTS	VARIETY MEANS
Armalaga	Plus	19.9	68.9	37.9	29.0	25.0	62.8	40.6	31.2
	Minus	12.0	39.6	20.4	26.4	18.1	13.8	21.7	
Bailey	Plus	19.9	41.1	38.0	41.1	16.8	38.5	32.6	32.7
	Minus	7.8	46.4	25.0	24.3	19.4	73.6	32.8	
Carman	Plus	22.0	28.7	34.5	41.8	27.6	68.8	38.9	36.1
	Minus	14.6	24.0	39.1	36.0	34.0	51.9	33.3	
Catawba	Plus	14.2	47.7	33.7	34.4	13.7	93.6	39.6	36.4
	Minus	14.0	57.6	42.0	34.6	17.8	33.2	33.2	
Concord	Plus	13.3	32.7	38.6	19.7	11.8	30.9	24.5	21.3
	Minus	13.4	18.8	38.5	7.4	8.4	22.0	18.1	
Delaware	Plus	25.0	64.3	25.5	36.0	13.6	77.0	60.2	46.6
	Minus	12.6	85.1	40.8	19.4	13.5	25.9	32.9	
Edna	Plus	17.8	26.2	46.7	28.8	20.5	14.3	25.7	26.5
	Minus	23.6	24.2	40.4	24.0	18.8	33.0	27.3	
Ellen Scott	Plus	20.3	74.3	53.0	40.8	35.2	68.4	48.7	41.6
	Minus	20.8	58.4	35.5	25.7	30.4	36.4	34.5	
Extra	Plus	38.5	55.9	38.8	28.2	17.3	41.1	36.7	36.6
	Minus	34.2	59.1	32.0	38.2	16.8	38.3	36.4	
Fern	Plus	39.0	22.8	37.9	26.0	22.9	34.5	30.5	27.5
	Minus	22.2	18.0	31.6	20.0	19.1	35.5	24.4	
Lenoir	Plus	36.2	46.4	45.7	29.3	17.1	50.8	37.6	32.4
	Minus	21.2	38.1	30.4	29.0	12.2	32.5	27.2	
Lucile	Plus	30.5	40.3	45.3	55.1	26.0	55.6	42.1	37.8
	Minus	30.8	41.6	36.8	35.4	12.2	44.1	33.5	
Portland	Plus	22.2	40.5	52.4	61.5	30.3	106.8	52.3	40.4
	Minus	17.0	39.9	26.6	29.0	22.6	35.0	28.4	
R. W. Munson	Plus	38.1	51.6	37.3	44.7	46.0	54.5	45.4	44.7
	Minus	34.0	47.0	35.7	44.7	49.7	52.4	43.9	
Means of sampling dates by treatments	Plus	25.5	45.9	40.4	36.9	23.1	57.0		
	Minus	19.9	42.7	33.9	28.2	20.9	37.7		
Means of sampling dates.....		22.7	44.3	37.2	32.5	22.0	47.3		

* Borax applied to plus-boron vines at rate of 10 pounds per acre in May, 1940.

TABLE 2—*Continued*

Mean of all plus-boron samples	38.1
Mean of all minus-boron samples	30.5
Difference between means necessary for significance (5 per cent level)	
(a) Between varieties	8.7
(b) Between sampling dates	5.6
(c) Between treatments	3.2
(d) Between variety-treatments	13.4
(e) Between sampling date-treatments	8.0

June 17 and September 11, 1942. Normally the fourth leaf from the shoot tip was taken. On actively growing vines the leaves at this point were young and about three-fourths mature size, but leaves of different samples were not comparable in this respect, because of boron deficiency, crop load, and seasonal conditions. Ten to fifteen leaves composed a sample. The same vines were sampled throughout the test. Other samples were obtained for a study of boron distribution in the current season's growth.

The analytical data are given in detail and in summary in table 2. The boron content of the leaves ranged from a low of 7.4 p.p.m. in the minus-boron Concord sample of September, 1941, to 106.8 p.p.m. in the plus-boron Portland sample of September, 1942. Variance analysis of the data showed significant differences for all three of the main effects; treatments, sampling dates, and varieties. Borax-treated vines had a higher boron content of the leaves than untreated vines. The boron content of the leaves was lowest in the early part of the growing season. The varieties Delaware, R. W. Munson, Ellen Scott, and Portland had the highest boron content with averages above 40 p.p.m., whereas Fern, Edna, and Concord had concentrations below 30 p.p.m. It was evident that boron content of the varieties was not necessarily in accord with varietal susceptibility to boron deficiency. Significant interactions between treatments, varieties, and sampling dates showed that the effect of the treatment upon boron content of the leaves was not the same throughout the season and that varietal differences also varied with date of sampling.

Although these significant differences are found in the analytical data, it is quite apparent that individual variety analyses give seemingly inconsistent results. A basis for this inconsistency may be found if both sampling technique and growth and yield performance of the individual vine are considered. The inability to obtain leaf samples of comparable age from the various vines has been mentioned. Many boron-treated vines producing heavy fruit crops ceased terminal growth in midsummer, while untreated vines without a crop continued in growth. The difference in fruiting, which was pronounced in most varieties, could very conceivably have affected the boron level in the terminal leaves.

The variable boron content of various parts of the shoot given in the following discussion shows clearly the necessity for more adequate sampling and consideration of the fruiting status of the vine.

Distribution of boron in the shoot

Representative shoots of the current season's growth of treated and untreated vines were sampled in an effort to determine the distribution of boron in the tissues. Vines of the Extra variety were sampled August 9, 1941, Carman vines June 17, 1942, and Lenoir vines May 5, 1943. The analyses are given in table 3.

The treated Extra vines had a slightly higher boron concentration than untreated vines, the greatest difference being found in the mature leaves about

TABLE 3

Distribution of boron in current season's shoots of the grape

VARIETY AND TIME OF SAMPLING	POSITION OF SHOOT ANALYZED	BORON CONTENT OF TISSUES	
		Boron-treated vines	Untreated vines
		$p.p.m.^*$	$p.p.m.^*$
Extra—sampled August 9, 1941. Light crop on untreated vines; heavy crop on treated vines	Old leaves adjacent to fruit clusters	26.0	24.8
	Mature leaves midway on cane	43.8	29.6
	Young leaves in active growth	33.7	30.0
Carman—sampled June 17, 1942. No crop and boron-deficiency symptoms on untreated vines in May and normal growth at sampling; heavy crop on treated vines	Basal leaves—below clusters	55.6	38.1
	Leaves adjacent to clusters	32.4	21.2
	Leaves above clusters	34.7	46.8
	Young terminal leaves	39.9	47.7
Lenoir—samples at full bloom May 5, 1943. No deficiency symptoms on untreated vines	Leaves below clusters	53.0	40.3
	Leaves adjacent to clusters	47.3	36.0
	Leaves above clusters	39.0	30.0
	Blossom clusters	38.6	34.6
	Stem below clusters	31.9	25.2
	Stem adjacent to clusters	27.6	17.5
	Stem above clusters	33.5	32.0

* On dry weight basis.

midway of the shoot. The old leaves of the shoots adjacent to the fruit clusters had the lowest boron content. The vines of both treatments were in active growth when sampled, but the treated vines had a much heavier crop of fruit.

The Carman vines, sampled when the fruit was developing, showed the lowest boron level in the leaves adjacent to the fruit clusters. The leaves on the basal half of the boron-treated vines were higher in boron than comparable leaves of the untreated vines, but the reverse was true of the leaves on the terminal half of the shoots. At the time of sampling, the untreated vines were in active growth with normal terminal leaves, but the leaves in the central portions of the cane showed

boron-deficiency symptoms.   Here again the treated vines were carrying a much
heavier fruit crop than the untreated.

A sampling more complete than that of the Extra and Carman vines was made
of the treated and untreated Lenoir vines at the time of full bloom.   On these
vines the shoots were divided into three sections, the part below the blossom
clusters, the part subtending the blossom clusters, and the part above the blossom
clusters, the stems, leaves, and flower clusters of the portions being analyzed
separately.   There was a progressive decrease in boron content of the leaves
toward the tip of the shoot, the boron content of the three portions in the treated
and untreated vines was 53.0, 47.3, 39.0 and 40.3, 36.0, 30.0 p.p.m., respectively.
The stem analyses also showed a higher boron content for the treated vines than
for the untreated, but positional differences were in different order from that of
the leaves, the highest boron level being in the terminal portion and the lowest
in the portion subtending the flower clusters.   The content of boron, in parts
per million, for the three stem portions of treated and untreated vines was 31.9,
27.6, 33.5 and 25.2, 17.5 and 32.0 respectively.   The boron content of the flower
clusters of the treated vines was 38.6 p.p.m., and the corresponding value for the
untreated-vine clusters was 34.6 p.p.m.

<div align="center">DISCUSSION</div>

It was apparent throughout the tests that the boron-deficiency symptoms
developed early in the growing season but failed to appear in later growth of the
shoot.   With severely affected vines the deficiency appeared as a stunting of the
new shoots shortly after growth commenced in the spring.   With less severely
affected varieties growth continued normally until after blossoming or set of
fruit and then symptoms suddenly appeared on the young leaves at the growing
shoot tips.   After failure to set fruit the cane resumed normal growth.   As a
result, shoots examined in the latter part of the growing season commonly showed
normal leaves at the base, then four or five leaves with deficiency symptoms,
then a continued growth of normal leaves.

This relationship between time of fruit set and the appearance of the deficiency
symptoms in the meristematic areas, accompanied by the failure of boron-defi-
cient vines to set fruit, strongly suggests a very close relationship between boron
nutrition and fruit setting of the grape.   Moreover, the effect upon set of fruit
was exhibited in instances where foliar effects were minor, if present at all, indi-
cating a possible specificity of the function of boron in fruit setting.   Whether
the boron effect is direct as an essential quantitative plant nutrient required in
definite amounts, or whether the action is that of a catalyst upon organic metab-
olism is not known.   Likewise, it would be of interest to know whether the ab-
scission of the flower is a direct result of boron deficiency in the cells in the pedicel
or whether abscission results from failure of a satisfactory gametic union caused
by lack of boron.   Armalaga, Catawba, and certain other varieties set partheno-
carpic fruits on the boron-deficient vines.   This phenomenon is observed with
many varieties under conditions of unfavorable fruit set, especially with Vinifera
varieties, where it is termed "millerandage."   Usually, however, only a few

seedless berries to a cluster are developed. Boron-deficient vines of Armalaga exhibited complete millerandage, developing full clusters of very small seedless berries. In this connection attention must be directed to the work of Oinoue (7), who obtained greatly increased fruit set on the variety Muscat of Alexandria by spraying with boron. This variety is rather commonly subject to millerandage, according to Winkler (11), who obtained an increased percentage of normal berries by reducing the crop in relation to vegetative area. Millerandage has been regarded for a long time as a possible nutritional trouble (4), but effective control by fertilizers has not been obtained. Snyder and Harmon (10) recently reported a remarkable improvement in fruit setting of Muscat of Alexandria by application of commercial zinc sulfate to the pruning cuts of vines not showing foliar symptoms of the little-leaf zinc deficiency. In this respect it is interesting to note that Schuster *et al.* (8) found commercial zinc sulfate to contain 15 p.p.m. of boron, and they suggested that this boron may be a beneficial factor in zinc sulfate treatments.

Is it possible that millerandage of grapes and the related physiological trouble, coulure, should be included in Atwater's (2) "ancient history" of boron symptoms?

The performance of the reflex-stamen or self-sterile varieties likewise shows a close relationship between boron nutrition and set of fruit. Physiological or nutritional factors have been recognized as probably partly responsible for self-sterility in many horticultural species. Fruiting of the self-sterile varieties of grapes is variable even under apparently comparable conditions for cross-pollination, differing widely with seasons, locations, and soils. Certain vineyards have been known to produce good crops of Brighton and Herbert regularly. The experiments reported suggest that the boron nutrition may be a strongly determinative factor in the fruiting of these varieties. It would seem well worth while to investigate the possibility of a relationship between the boron nutrition and fruit set of other horticultural species exhibiting peculiar sterility problems.

It has been pointed out that boron-deficiency symptoms seldom developed in new growth produced during the latter part of the growing season. It is possible that during this period the relationship between the demand for boron by the actively growing tissues and the ability of the soil and root system to supply boron is more favorable than that existing in the early part of the season when growth is extremely rapid over all parts of the vine. If we consider boron as essential especially in meristematic tissues, then the tendency toward exhibition of systems in the period of rapid growth expansion is quite logical. It must also be assumed, then, that the vine is unable to store sufficient boron in the trunk and roots and transfer it to the growing points at an adequately rapid rate for the early season growth.

The most important practical value of a series of plant tissue analyses of a particular mineral nutrient is the establishment of optimum and critical levels of that nutrient in relation to deficiency symptoms and plant growth. In the present work, although borax application to the soil resulted in consistent and positive correction of abnormal fruiting and growth condition and, in doing so,

generally increased the boron content of terminal leaves on the canes, the data obtained in analyses of these leaves do not permit accurate definition of deficiency levels of boron. Several factors must be considered. First, the analyses obtained, corroborated later by the results of sand culture experiments, make it questionable whether the terminal leaves of the cane afford the best indication of the boron status of the vine. Second, with the exception of the samples of May 1, 1941, no series of samples were taken at a time when the vines were actually developing symptoms of boron deficiency in the terminal leaves. The critical period for development of symptoms was found to be shortly after growth inception in the spring and at time of fruit-setting; thereafter, all vines, both treated and untreated, generally failed to show deficiency symptoms in the later growth. Furthermore, many of the samples taken consisted of mature leaves or leaves not in active stage of growth. Since the symptoms of boron deficiency can only become apparent if the deficiency occurs when the leaves are in active meristematic condition, it is possible that such leaves may exhibit a very low boron level but no outward symptoms of deficiency, or likewise the converse may be true.

The effect of blossoming, fruit set, and fruit development upon the boron level in the vine in relation to the occurrence of deficiency symptoms is not clear. The flower clusters of Lenoir had a boron content comparable to that of the accompanying leaves. On the other hand, the leaves subtending the fruit clusters on both treated and untreated vines of Carman and Extra showed lower boron content than leaves on other portions of the cane. Does this indicate mobilization and a utilization by the fruit cluster of the boron in the adjacent leaves? As possible further evidence of the demand of fruit development on the boron supply, is the lower level of boron found in the terminal portions of the heavily fruiting plus-boron Carman canes, than that found in comparable portions of low-yielding untreated canes.

Within the past year, in greenhouse studies, young vines in sand cultures without boron in the nutrient solution have exhibited the same deficiency symptoms as those found in the field. More detailed analyses of these vines have given evidence concerning the mobility of boron within the vine and its relation to development of the deficiency in localized parts of the vine. These data will be presented in a later paper.

SUMMARY

Application of borax to the soil at the rate of 10 pounds to the acre resulted in positive correction of abnormal growth and fruiting of a number of grape varieties in a vineyard on Norfolk sandy soil.

Foliar symptoms of the boron deficiency developed early in the growing season, particularly just after development of blossom clusters.

Late-season growth seldom developed deficiency symptoms.

Vines showing boron-deficiency symptoms formed blossom clusters but set very few fruits. Fruit yield also was severely affected on some vines which showed little evidence of deficiency symptoms in the foliage.

Certain varieties, in particular Armalaga, exhibited millerandage, that is, they set parthenocarpic or seedless fruits on boron-deficient vines.

Borax greatly increased the set of fruit of self-sterile or reflex-stamen varieties.

Varieties differed widely in their susceptibility to boron deficiency.

Borax application increased the boron content of the leaves.

Boron content of leaves was lowest in the early part of the growing season.

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NUTRITION OF BRASSICA AND POTATOES

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BRASSICA STUDIES

The symptoms of boron deficiency in some plants of the genus *Brassica* were published in 1941 (2), and therefore only a few brief statements of the symptoms are presented here. The most conspicuous symptom of boron deficiency in broccoli is curling and rolling of the leaves. The heads have brown buds which fall off prematurely. Swelling or cork on the petiole is frequently observed. Cabbage receiving an inadequate supply of boron also has deformed leaves and swellings on the petioles, and the leaves of the head frequently are not attached to the stem. Boron deficiency in cauliflower is first expressed as curling and rolling of the leaves; later, watery and brown areas appear on the inside of the stems and on the head. The leaves of boron-deficient chinese cabbage are rugose with occasional chlorotic areas, and the petiole is cracked crosswise. Leaves of deficient kale plants are chlorotic. The surface of the bulb of boron-deficient kohlrabi is covered with cork, and the inside may have watery or brown areas. The first external symptoms of boron deficiency of rutabagas and turnips are curled, rolled, and rugose leaves; later, the surface of the root is usually coarsely russeted.

The seeds of cauliflower do not contain enough boron properly to develop the cotyledons (2). The total fresh weight of plants grown with 0.3 p.p.m. of boron was greater than the weight of plants grown with 0.1 and 0.5. Plants inadequately supplied with boron were dwarfed, the edible part of the plant being reduced the most, and all other organs of the plants were in the same mass relationship as in healthy plants (root-top ratio of healthy and deficient plants are the same). Some boron appeared to be translocated from the leaves of deficient plants before they fell off.

Experimental methods

To study the effect of elements other than boron on boron nutrition of *Brassica*, seeds were planted in sand and supplied with nutrient solution containing a low level of boron until the seedlings were transplanted to sand supplied with the test solution by the continuous flow method. In all but one of the experiments the factorial design of experiment was used. The test plant in two of the experiments was broccoli and in the other kale. At harvest the data were analyzed statistically, and odds of 19 to 1 were considered significant.

First broccoli experiment

Broccoli was grown in four different nutrient solutions selected from twenty-two which had been used by various investigators to study boron deficiency.

The solutions, designated A, B, C, and D, were those used by Hill and Grant (10), by Hoagland and Snyder (11), by Johnson and Dore (13), and by Shive (18).

The nutrient solutions used in the first broccoli experiment are described in table 1. With continuous flow supplying 2 liters of nutrient solution per plant daily, the osmotic pressure after the solution had been used was higher than before, and the selective absorption of the roots caused the pH of all solutions to change.

TABLE 1

Nutrient solutions used in the broccoli experiment

SOLUTION	OSMOTIC PRESSURE		pH		SALT CONTENT		RATIO OF ELEMENTS				
	Fresh	Used	Fresh	Used			N	P	K	Ca	Mg
					gm./l.	p.p.m.					
A.............	.61	.69	4.8	6.7	1.94	593	3	1	1.5	4	1
B.............	.62	.63	4.8	8.0	2.31	787	4	0.6	4.7	4	1
C.............	.48	.58	5.0	6.5	1.41	586	1	1*	1	1	1.4
D.............	.65	.92	5.3	6.9	1.65	590	6	0.3	2.5	1	0.1

* Phosphorus of this solution was taken as unity. Its absolute value is 49.6 p.p.m.

TABLE 2

Harvest data in the first broccoli experiment

Averages of 12 plants

SOLUTION	FRESH WEIGHT			PLANT HEIGHT	LEAF LENGTH	RATIO OF LEAF LENGTH/ WIDTH	NUMBER OF BORON- DEFICIENCY SYMPTOMS**
	Head	Suckers	Total*				
	gm.	gm.	gm.	in.	in.		
A..................	158	46	1020	25.2	22.4	2.6	6.5
B..................	90	310	1655	23.7	26.0	2.7	3.3
C..................	40	25	673	19.1	21.2	2.9	0.8
D..................	24	42	571	17.1	18.3	2.2	0.6
Difference necessary for significance....	114	111	575	4.4	2.7		4.5

* This includes stem, leaves, head, and suckers.
** Boron-deficiency symptoms counted were rolled leaves, curled leaves, swelling on the midrib, swellings on the petiole, cork on the petiole, cork on the stem, split petioles, watery areas in the stem, brown areas in the stem, and absized blossom buds.

The measurements obtained at harvest are presented in table 2, each of the figures being an average of 12 plants. Nutrient solution A produced the largest heads and the tallest plants. Plants supplied with solution B had the greatest total weight (stem, leaves, suckers, and head). This solution, however, produced a large number of suckers over the entire plant, which may explain its inefficient production of heads. The C solution produced plants which were inferior to those grown in either the A or the B solution. The plants in the C

solution appeared very different from those grown in other solutions, having narrow upright leaves covered with a very heavy bloom. Solution D produced the poorest plants, plants which were not healthy during any part of their growth.

The boron deficiency of broccoli in this experiment varied with the composition of the nutrient solution, being more common and more severe in solutions with high calcium.

Kale experiment

Another experiment was planned to vary at three levels the quantity of nitrogen, potassium, calcium, and magnesium in order to study further the effect of these elements on boron deficiency. The levels of the four elements were selected to obtain as nearly as possible the same absolute amount of each element as was used in the preceding experiment. Nitrogen was at the levels of A, C, and D; phosphorus was supplied at the same level as in solution C; potassium was equivalent to C and D and also was supplied at a higher level; calcium and magnesium were supplied at the same concentrations as A, B, and C, with one higher concentration of each element. In selecting levels above the absolute amount used in the first experiment, the ratio of two elements was given consideration; for example, the high potassium was set at 369.1 p.p.m. because this would give approximately the same K/P ratio as that in both solutions B and D of the preceding experiment. The different ratios used in this experiment were as follows (1 = 49.6 p.p.m.):

N	P	K	Ca	Mg
1.2	1.0	1.3	1.3	1.0
2.8	1.0	2.5	4.0	1.4
5.9	1.0	7.4	12.1	2.6

All of the 81 possible combinations were studied, on kale as the test plant, and boron was supplied at 0.05 p.p.m. in all solutions. The first plant to show boron deficiency received low N and Ca, medium Mg, and high K (1–1–7–1–1.4). A number of the plants receiving high calcium had very brittle petioles, yet at harvest only 10 of the 81 plants showed boron-deficiency symptoms, and statistical analysis did not indicate association between any level of any of the elements studied and the deficiency.

Second broccoli experiment

Another experiment was conducted, with broccoli as the test plant, and potassium, calcium, and boron were varied as follows (1 = 50 p.p.m.):

N	P	K	Ca	Mg	S	Cl
5	6	1	1	1	2	2
5	6	4	4	1	2	2
5	6	8	8	1	2	2

This study was conducted in three kinds of sand. The results of this experiment show that the levels of boron studied, 0.01, 0.5, and 1.5 p.p.m., did not affect fresh weight or the number of boron-deficiency symptoms (table 3). The fresh

weight was significantly increased with increasing amounts of calcium, and the low level of calcium resulted in more boron deficiency than the high level. Potassium did not affect fresh weight or the number of boron-deficiency symptoms. The sand obtained from a lake shore in Maine produced a greater yield than the fine sand and more boron-deficiency symptoms than either the fine or the coarse sand. These results are in contrast to those obtained in previous experiments, in which no difference was observed with these three sands.

TABLE 3

Harvest data in the second broccoli experiment

TREATMENT	MEAN FRESH WEIGHT	MEAN NUMBER OF BORON-DEFICIENCY SYMPTOMS
	gm.	
Lake sand..	142.1	5.0
Fine sand..	97.8	2.9
Coarse sand...	134.9	3.9
0.01 p.p.m. Boron.................................	105.2	4.1
0.5 p.p.m. Boron..................................	134.4	3.3
1.5 p.p.m. Boron..................................	135.2	4.4
50 p.p.m. Calcium................................	78.1	4.6
200 p.p.m. Calcium...............................	116.0	4.3
00 p.p.m. Calcium................................	180.7	3.0
50 p.p.m. Potassium..............................	103.7	4.3
200 p.p.m. Potassium.............................	136.3	3.6
400 p.p.m. Potassium.............................	134.8	4.0
Difference necessary for significance..............	34.03	1.32

Discussion

Midgley and Dunklee (14), Cook and Millar (6), and Parks and Shaw (16) have suggested that chemical fixation of boron is brought about by the calcium ion. The failure to develop boron-deficiency symptoms in the kale and second broccoli experiments in which there were 26 replicates of each level of calcium in each experiment shows the absence of any such simple chemical fixation. Purvis (17) has shown that if boron is limiting, the deficiency symptoms may be brought out by copper, magnesium, and manganese. Probably many other factors are involved, and unless all these are included in one experiment it will be difficult, if not impossible, to study the effect of elements other than boron on boron nutrition.

POTATO EXPERIMENTS

Stem-end browning is a disease of potato tubers prevalent in Maine. Many unsuccessful attempts have been made to isolate a pathogenic organism, and

cultural practices and fertilizer treatments have not satisfactorily controlled it. The stem ends of the tubers become discolored as a result of a necrosis primarily in the xylem and phloem. Usually this discoloration does not extend over a half inch into the tuber. Folsom (8) has shown that the development of the discoloration usually reaches its peak after 100 days of storage at about 52°F.

In the experiments reported here, small seed pieces of potatoes were sprouted in sand supplied with Hoagland's nutrient solution. When they were well rooted they were transplanted to sand supplied with nutrient solution by continuous flow. Three cultures of each of 31 nutrient solutions were used. The regular nutrient solution had the following composition, in parts per million: N 400, P 397, K 500, Ca 500, Mg 120, S 100, Cl 0, Cu 0.1, and B 0.05. The concentration of the solution was varied for some cultures, and the amount of N, K, Ca, Mg, and S was $\frac{1}{4}$, $\frac{1}{2}$, 2, and 3 times that of the regular solution, whereas B was supplied at 0.01 and 0.001 p.p.m. In some of the cultures, Cl was added (125, 250, 500, and 750 p.p.m.). Copper was supplied at 25 and 50 p.p.m., and one culture received 100 p.p.m. after the plants were well developed.

After storage of over 100 days at 52° F., none of the tubers from the regular solution or the solution with half the regular nitrogen showed any discoloration. Typical symptoms of stem-end browning were developed in some of the tubers receiving half the regular concentration, twice the regular nitrogen, three times the regular potassium, half the regular calcium, three times the regular sulfur, 100 p.p.m. of copper applied late, and 500 p.p.m. of chloride. Stem-end browning was most frequently observed in the tubers grown in the solution with low boron (0.01 and 0.001 p.p.m.). In addition, some of these tubers showed a more severe type of boron deficiency which developed in storage (fig. 1). The skin developed a thick, corky russeting, particularly near the stem end, and the skin of some was a brownish black. When the tubers were cut, the tissue immediately below the dark skin was found to be necrotic and to extend to the vascular ring or deeper. No symptoms of boron deficiency were observed on the tops of plants grown in the greenhouse. These results indicate that stem-end browning is the first symptom of boron deficiency in potato tubers and that with a greater deficiency the skins are conspicuously russeted and/or the tissue just below the skin is necrotic.

Folsom and Rich (7) in a field experiment in Maine showed that 1, 2.5, 5, and 10 pounds of boron per acre had no effect on potato growth, yields, or stem-end browning. Under similar conditions, Chucka and Hawkins (3) showed no increase in yield, and Chucka (4) showed a slight reduction in yield a few years later. On the other hand, Harrington (9) found that limited amounts of boron promoted earlier growth, increased the yield, and improved the quality of potatoes in some sections of Montana. In Pennsylvania (1) 10 pounds of borax per acre increased the yield of potatoes. It has been shown (12, 14, 15, 16, 17, 19) that applications of K, Ca, and Mg to the soil[2] may induce boron deficiency in

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[2] In this number of SOIL SCIENCE, Jones and Scarseth give their views on the calcium-boron interrelationship, and Reeve and Shive present information on the potassium-boron and calcium-boron relationships.

some crops. The potato section of Maine has always used liberal applications of potassium, and the use of lime and magnesium has been greatly increased during the last 10 years. Furthermore, data from the permanent fertilizer plots in Aroostook County, Maine, indicate that high applications of potassium and of calcium result in increases in the amount of stem-end browning (5). It there-
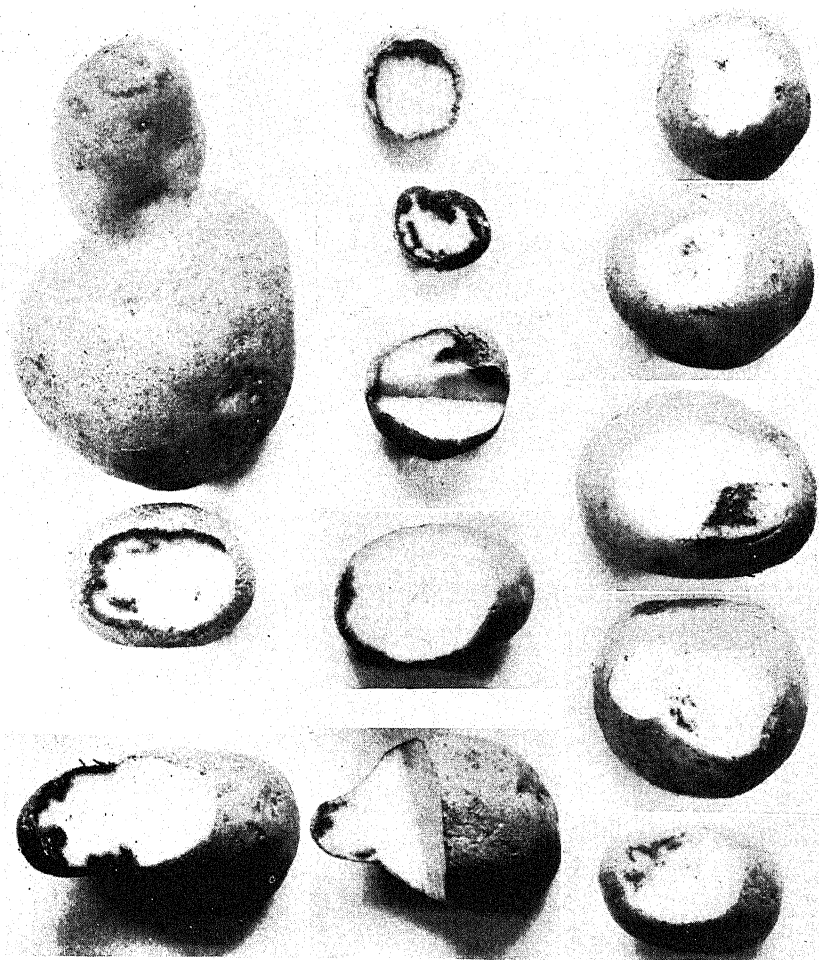
FIG. 1. BORON DEFICIENCY AND STEM-END BROWNING OF POTATO TUBERS

The left and center columns show boron deficiency, the right column shows stem-end browning. All tubers are from plants that received 0.001 p.p.m. of boron.

fore appears that stem-end browning may be a case of mild boron deficiency which does not express itself in the leaves and may not alter the yield in every type of soil every season. As responses to soil applications of boron have not been observed in Maine, it may be necessary to spray the boron on the plants growing in some types of soils and with some methods of fertilizer application.

SUMMARY

High-calcium nutrient solutions (three replicates) produced more boron deficiency than did low calcium in broccoli plants. In two experiments, however, with kale and broccoli, in which 26 replicates of each level of calcium were tried, there was no significant association of high calcium and boron deficiency. Three levels of nitrogen, potassium, and magnesium had no significant effect on boron-deficiency symptoms.

Stem-end browning of potato tubers was produced in the greenhouse on low boron, and a more severe symptom of boron deficiency in the tubers is described.

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THE IMPORTANCE OF BORAX IN LEGUME SEED PRODUCTION IN THE SOUTH[1]

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The profitable production of legume seed may be influenced by many factors. Among these, soil fertility; climatic factors, such as temperature, humidity, and rainfall; pollination; and selection and breeding for an increase in ability to produce good seed sets, have been investigated. It is now commonly accepted that most legumes have a relatively high requirement for boron. In many instances low boron supply in the soil has been responsible for low yields, or even failure of many legume crops.

Boron deficiency has been observed on many crops and under varied soil conditions in North Carolina for a number of years. In the last few years the expansion of legume crops has been emphasized. This expansion is desirable, particularly in the South, for soil improvement and development of a sound livestock program. In recognition of the importance of boron in plant growth and of legumes in southern agriculture, studies were initiated to ascertain the boron status of the soils in the state; and the effects that added boron might have upon the growth of legume hay and seed crops. This paper deals principally with the effects of boron on the seed production of alfalfa, vetch, crimson clover, peanuts, and soybeans.

REVIEW OF THE LITERATURE

It has been observed in a number of instances and under varied soil conditions (2, 5, 6, 8, 9, 12) that boron is essential in the production of alfalfa seed. Naftel (10) determined that 0.3 p.p.m. B was most effective in the development of crimson clover heads and total plant yields. Dmitriev (7) found that seed production of red clover was raised by the addition of boron to limed soils. Sokolov and others (13) also observed an increase of the seed yield of leguminous plants on podzol soils when much lime was introduced together with boron. Boron applications are recommended, according to Bobko (5), for the seed production of clover and lucerne on limed podzol soils. Wester and Magruder (16) found that boron significantly increased the yield of dry lima beans on Elsinboro sandy loam soil.

Nowotnowna (11), working with soybeans, reported a yield of six times more beans and with a higher oil content when supplied adequate boron.

From tests conducted in Alabama, Albrecht (1) concluded that some increase in seed yield of hairy vetch can be expected when boron is applied to a light, sandy Norfolk soil.

There appears to be no reference in the literature to the value of boron in the growth of peanut fruit.

AVAILABLE BORON IN NORTH CAROLINA SOILS

Soil samples were collected from 93 of the 100 counties in North Carolina representing the two major soil types of agricultural importance in each county, and representing all types of agriculture found in the state. These soils were analyzed for available boron by the method of Berger and Truog (4). The results show that the supply of available soil boron may be too low for the most economic production of many legume crops as well as their seeds. Typical data in tables 1 to 3 show the available boron contents of several soil types from the Coastal Plain (table 1), Piedmont (table 2), and Mountain (table 3) sections.

EFFECT OF BORON ON ALFALFA SEED PRODUCTION

During the fall and winter of 1940–41 a study was initiated on 13 outlying alfalfa fields in the Piedmont and Mountain sections of the state to ascertain the value of borax in the growth of alfalfa hay. Duplicate $\frac{1}{40}$-acre plots were established, and borax additions were made to supply 0, 0.5, 1, and 1.5 p.p.m. boron, fertilization being uniform. The use of boron resulted in hay yield increases as high as 27 per cent. Three of the field locations in the Piedmont were selected in the spring of 1942 to ascertain what effect the previous boron additions might have on seed development. Results are shown in figure 1. At the second cutting of hay, strips ($\frac{1}{435}$ acre) were left on the several plots to allow seed to mature. The seeds were harvested by hand, rubbed out on a screen, and cleaned with a small laboratory seed cleaner. Borax increased both hay and seed yields. The average seed yield for the check plots was 19.1 pounds per acre. The addition of 0.5, 1, and 1.5 p.p.m. boron produced an average of 460, 433, and 529 per cent increase respectively. The effects of boron additions on increase in individual field yields of hay and seed are shown in table 4, and the boron contents of hay and seed are shown in table 5. From the germination data given in table 6 there seems to be no consistent difference in the germination quality of the seed produced at the various boron levels.

EFFECT OF BORON ON SEED PRODUCTION OF HAIRY VETCH

Vetch has long been recognized as an important winter legume in the South, both from the standpoint of a hay crop and as a green manure. Attempts to produce locally grown seed have met with failure, and as a result shipments from the Pacific Northwest are common. Data from the Alabama Agricultural Experiment Station indicate that boron applied to a light, sandy Norfolk soil low in available boron may make it possible to produce vetch seed on southern

TABLE 1
Available boron* in some Coastal Plain soils of North Carolina

SOIL TYPE	COUNTY	pH OF TOP-SOIL	AVAILABLE BORON	
			Topsoil†	Subsoil‡
			p.p.m.	*p.p.m.*
Norfolk sand	Bladen	5.67	0.20	0.15
	Harnett	5.31	0.15	0.13
	Johnston	5.47	0.11	0.18
	Moore	4.95	0.29	0.09
	Sampson	5.65	0.20	0.10
Norfolk fine sand	Chowan	5.55	0.29	0.29
	New Hanover	5.55	0.16	0.12
	Onslow	5.27	0.28	0.11
Norfolk fine sandy loam	Duplin	4.78	0.23	0.13
	Edgecombe	5.40	0.40	0.20
	Lenoir	5.95	0.22	0.09
	Martin	6.50	0.21	0.14
	Pender	5.00	0.10	0.10
	Pitt	5.50	0.20	0.20
	Wayne	5.40	0.20	0.10
Norfolk sandy loam	Wilson	5.35	0.22	0.18
	Wayne	5.12	0.20	0.10
	Scotland	5.60	0.30	0.20
	Robeson	5.10	0.10	0.10
	Lee	5.28	0.30	0.12
	Johnston	5.50	0.12	0.11
	Edgecombe	5.98	0.20	0.20
Dunbar very fine sandy loam	Beaufort	5.30	0.20	0.10
	Bertie	4.98	0.34	0.31
	Onslow	5.00	0.15	0.13
Bladen loam	Beaufort	4.34	0.30	0.45
	Hyde	3.95	0.33	0.28
	Washington	4.52	0.20	0.10
Coxville fine sandy loam	Hertford	5.51	0.20	0.20
	Martin	4.70	0.27	0.10
Portsmouth sand	Bladen	4.60	0.25	0.15
Portsmouth sandy loam	Robeson	5.10	0.20	0.10
Portsmouth fine sandy loam	Carteret	4.75	0.40	0.20
	Chowan	5.80	0.29	0.29
	Craven	4.60	0.30	0.20
	Duplin	5.30	0.22	0.11
	Jones	5.20	0.27	0.18
	Pender	5.00	0.30	0.15
	Pitt	4.85	0.20	0.20
Peat	Camden	5.37	0.43	0.40
	Washington	4.15	0.40	0.35
Average available boron...................................			0.24	0.13

* Hot water soluble.
† 0–6 inches.
‡ 6–12 inches.

TABLE 2

Available boron* in some Piedmont soils of North Carolina

SOIL TYPE	COUNTY	pH OF TOPSOIL	AVAILABLE BORON	
			Topsoil†	Subsoil‡
			p.p.m.	*p.p.m.*
Cecil sandy loam	Caswell	4.84	0.30	0.20
	Catawba	4.83	0.30	0.20
	Davidson	5.23	0.24	0.29
	Gaston	5.25	0.21	0.10
	Iredell	6.70	0.05	0.00
Cecil fine sandy loam	Lincoln	4.95	0.30	0.20
Cecil coarse sandy loam	Franklin	5.40	0.30	0.23
Cecil gravelly loam	Anson	5.20	0.22	0.18
Cecil clay loam	Cabarrus	7.85	0.50	0.30
	Catawba	4.50	0.30	0.20
	Cleveland	5.10	0.30	0.25
	Gaston	5.30	0.10	0.11
	Iredell	6.32	0.30	0.20
	Lincoln	6.38	0.50	0.40
	Rowan	4.40	0.30	0.28
	Surry	5.20	0.29	0.18
	Yadkin	5.67	0.45	0.30
Cecil clay	Alamance	5.80	0.29	0.14
Durham sandy loam	Alamance	5.65	0.18	0.18
	Warren	6.10	0.32	0.36
Georgeville stoney silt loam	Montgomery	4.32	0.37	0.23
Georgeville gravelly silty clay	Chatham	5.50	0.30	0.20
Georgeville gravelly silt loam	Union	5.30	0.27	0.18
Georgeville gravelly silty clay loam	Person	5.17	0.34	0.18
Georgeville silt loam	Anson	4.87	0.22	0.20
	Durham	4.25	0.13	0.26
	Randolph	4.95	0.30	0.20
Iredell sandy loam	Caswell	5.10	0.30	0.20
Iredell fine sandy loam	Rowan	5.34	0.20	0.10
Iredell loam	Cabarrus	6.32	0.35	0.20
Alamance silt loam	Granville	5.30	0.15	0.07
	Randolph	4.53	0.05	0.00
	Union	5.10	0.28	0.19
Alamance gravelly silty loam	Chatham	5.16	0.30	0.20
Appling coarse sandy loam	Franklin	4.94	0.23	0.20
Appling sandy loam	Warren	6.47	0.24	0.12
Average available boron..................................			0.27	0.20

* Hot water soluble.
† 0–6 inches.
‡ 6–12 inches.

soils. The yields, as given in table 7, though low, do indicate that the addition of boron had a marked effect on seed yield.

TABLE 3

Available boron* in some mountain soils of North Carolina

SOIL TYPE	COUNTY	pH OF TOPSOIL	AVAILABLE BORON	
			Topsoil†	Subsoil‡
			p.p.m.	*p.p.m.*
Ashe loam	Alleghany	6.00	0.30	0.10
	Caldwell	4.43	0.30	0.13
	Watauga	6.40	0.42	0.28
	Yancey	5.10	0.40	0.30
Porters stoney loam	Cherokee	4.45	0.40	0.35
	Haywood	5.60	0.50	0.30
Porters sandy loam	Transylvania	5.70	0.30	0.10
Porters loam	Alleghany	5.80	0.20	0.10
	Caldwell	5.40	0.44	0.13
	Swain	5.50	0.48	0.38
	Transylvania	5.56	0.40	0.15
	Watauga	5.10	0.48	0.20
	Wilkes	6.40	0.48	0.31
	Yancey	6.50	0.30	0.40
Talladega silt loam	Cherokee	4.40	0.30	0.20
Hayesville silt loam	Clay	5.57	0.24	0.19
Hayesville loam	Madison	5.40	0.30	0.31
Hiawasse silt loam	Clay	4.57	0.15	0.25
Hiawasse sandy loam	Swain	5.40	0.32	0.50
Surry loam	Surry	6.05	0.28	0.20
Average available boron............................			0.35	0.29

* Hot water soluble.
† 0-6 inches.
‡ 6-12 inches.

EFFECT OF BORON ON PEANUT GRADE QUALITY

Grade quality and yield are important factors in the economic production of peanuts. Low yields and poor grade quality have been attributed to various causes. They are often associated with low calcium and potassium supply. The possibility that low boron supply in the soil could also be a cause of low quality in peanuts led to investigation of this problem.

FIG. 1. EFFECT OF BORON FERTILIZATION ON ALFALFA SEED HEADS
Left, No boron applied.   Few or no seed heads developed.
Right, 1 p.p.m. boron applied.   Normal seed head growth and development.

TABLE 4

*Effect of boron on yields of alfalfa hay and seed at three locations in North Carolina
in 1942*

Yields in pounds per acre

BORON ADDI-TIONS TO THE SOIL	HAY YIELDS			SEED YIELDS§		
	Location A*	Location B†	Location C‡	Location A	Location B	Location C
p.p.m.						
0.0	7,077	4,903	9,880	17.9	9.2	30.1
0.5	7,737	5,307	10,303	133.9	58.0	132.1
1.0	7,649	5,548	11,295	131.6	64.0	109.7
1.5	7,246	5,472	11,126	133.5	54.2	172.7

* Location A—Caswell County, Appling sandy loam, 0–6 inches; pH 6.65; exchange capacity, 5.25, exchangeable Ca, Mg, and K, 3.68, 1.71, and 0.27 m.e./100 gm. respectively (3); available P, 11.2 p.p.m. (14); organic matter, 2.57 per cent (15); available boron, 0.39 p.p.m.

† Location B—Davidson County, Georgeville clay loam, 0–6 inches; pH 7.10; exchange capacity, 9.4, exchangeable Ca, Mg, and K, 5.12, 1.92, and 0.86 m.e./100 gm. respectively; available P, 17.86 p.p.m.; organic matter, 1.79 per cent; available B, 0.17 p.p.m.

‡ Location C—Davidson County, Cecil sandy loam, 0–6 inches; pH 7.45; exchange capacity, 4.3, exchangeable Ca, Mg, and K, 4.64, 0.90, and 0.33 m.e./100 gm. respectively; available P, 196 p.p.m.; organic matter, 1.52 per cent; available B, 0.11 p.p.m.

§ Seed yields from location C single plots only—difference in seed yields between treatments must be 48.35 pounds for significance.

In the spring of 1942 seventeen field locations that were to be planted to peanuts were selected in the Coastal Plain region.   The soils selected were of a sandy type similar to those listed in table 1, which were found to be low in available

boron. Borax was applied broadcast at a rate of 5 pounds per acre at planting time to a ¼-acre area, an adjacent area being used as a check. No visual differ-

TABLE 5

Effect of boron additions to the soil on boron content of alfalfa hay and seed

Boron contents, oven-dry basis

BORON ADDI-TIONS TO THE SOIL	BORON CONTENT OF HAY*			BORON CONTENT OF SEED		
	Location A†	Location B	Location C	Location A	Location B	Location C
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0.0	15.0	15.0	15.0	6.0	10.0	15.0
0.5	27.5	25.0	28.8	15.0	20.0	20.0
1.0	32.5	30.0	30.0	20.0	20.0	20.0
1.5	31.3	30.0	32.5	20.0	20.0	20.0

* Boron contents given on second-cutting hay, or growth that produced the seed.
† See footnote table 4 for soil analyses at locations A, B, and C.

TABLE 6

Germination of alfalfa seed grown under different boron levels

BORON ADDI-TIONS TO THE SOIL	GERMINATION			HARD SEED		
	Location A*	Location B	Location C	Location A	Location B	Location C
p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.0	85.0	91.0	74.0	5.5	2.5	8.0
0.5	91.0	91.0	73.0	3.0	3.0	6.5
1.0	92.0	80.0	84.0	5.5	5.5	7.5
1.5	86.0	82.5	82.0	3.5	5.0	4.0

* See footnote table 4 for soil analyses at locations A, B, and C.

TABLE 7

Influence of boron on seed production of hairy vetch*

Applications and yields in pounds per acre

TREATMENT NUMBER	FERTILIZER APPLICATIONS				SEED YIELD†	INCREASE OVER NO-FERTILIZER PLOT (NO. 1)
	Slag	Muriate	Dolomite	Borax		
1	0	0	0	0	42.8‡	...
2	0	0	0	20	72.2	29.4
3	800	50	...	0	69.8	27.0
4	800	50	...	20	83.2	40.4
5	...	50	400	0	75.7	32.9
6	...	50	400	20	112.0	69.2

* Unpublished data of H. R. Albrecht, Ala. Agr. Exp. Sta., Auburn, Ala.
† Average of three replications.
‡ Difference in yields between treatments must be 24.04 pounds for significance.

ences were noted in growth during the summer between the check and the borax-treated areas. At time of harvest, 25 plants from each of the check and borax-treated areas were carefully removed at random from the soil with a hay fork.

After air-drying for 30 days, the nuts were removed from the plants by hand. Shelling percentage and grade sizes of the nuts were determined. From the average of results shown in table 8 it is apparent that borax reduced shelling percentage slightly and affected grade quality significantly. On an average, borax decreased the percentage of small nuts slightly, significantly decreased medium nuts, and significantly increased large nuts.

TABLE 8

Effect of borax on shelling percentage and grade quality of peanuts

COUNTY	FIELD NUMBER	VARIETY	DIFFERENCE IN SHELLING PERCENTAGE DUE TO BORAX	INCREASE OR DECREASE IN NUT SIZES DUE TO BORAX		
				Large*	Medium†	Small‡
				per cent	*per cent*	*per cent*
Bertie	1	Runner	−0.5	+5.83	−5.61	−0.22
	2	Bunch	−0.8	+9.27	−8.37	−1.35
Chowan	3	Jumbo Runner	−0.0	+4.67	−4.54	−0.13
	4	Jumbo M. Runner	+3.2	−4.58	+1.10	+3.48
Gates	5	Bunch	+0.5	+1.84	−0.65	+2.49
	6	Runner	+2.9	+1.13	−0.71	+1.84
	7	Bunch	−0.3	−3.43	−2.92	−0.51
	8	Bunch	+0.6	−1.51	+1.03	+0.48
Hertford	9	Bunch	−0.8	+7.43	−6.95	−0.48
	10	Bunch	−3.5	+22.02	−13.17	−8.85
	11	Bunch	−11.4	+2.36	−10.59	+8.23
	12	Runner	+1.3	+13.06	−9.53	−3.53
Martin	13	Jumbo	−0.6	+13.16	−3.16	−10.00
	14	Bunch	−2.2	+16.41	−9.81	−6.57
	15	Small Runner	−0.7	−2.69	+2.53	+0.16
Northampton	16	Bunch	+0.6	−1.51	+1.03	+0.48
	17	Bunch	+4.5	+17.13	−9.08	−8.05
Average			−1.78	+6.15	−4.74	−1.47
Difference required for significance (.05)			1.75	4.05	2.64	2.42

* Nuts remaining on a screen $\frac{20}{64}$- by 1-inch perforations.

† Nuts remaining on a screen $\frac{14}{64}$- by 1-inch perforations.

‡ Nuts passing a $\frac{14}{64}$- by 1-inch perforation.

EFFECT OF BORON ON CRIMSON CLOVER SEED PRODUCTION

Crimson clover was seeded at the rate of 25 pounds per acre on September 15, 1942, at the College Dairy Farm on Cecil sandy loam soil. The soil was sampled to a depth of 6 inches, and is characterized as follows: pH, 6.4; exchange capacity, 3.9; exchangeable Ca, Mg, and K, 2.06, 0.70 and 0.37 m.e./100 gm. respectively; available P, 81.66 p.p.m.; organic matter, 1.93 per cent; available boron, 0.39 p.p.m. The treatments involved 0 and 15 pounds of borax per acre each. Plots were replicated four times. An application of 300 pounds per acre of a 2–10–6

fertilizer was made to all plots at the time of seeding. Good stands were obtained on all plots.

The no-borax series produced an average of 270.5 pounds of cleaned seed per acre, whereas the series receiving 15 pounds borax per acre produced an average of 390.5 pounds, or an increase of 120 pounds of seed, which was significant. No differences were noted in the germination quality of the seed produced. The boron content of the seed (oven-dry basis) produced under no-borax treatment was 4.5 p.p.m., and that of seed under borax treatment, 6.5 p.p.m.

The increase in yield of crimson clover seed (fig. 2) due to borax addition is attributed to the growth of a more vigorous type seed head and the growth of fully matured, plump seed in the apical portion of the head.
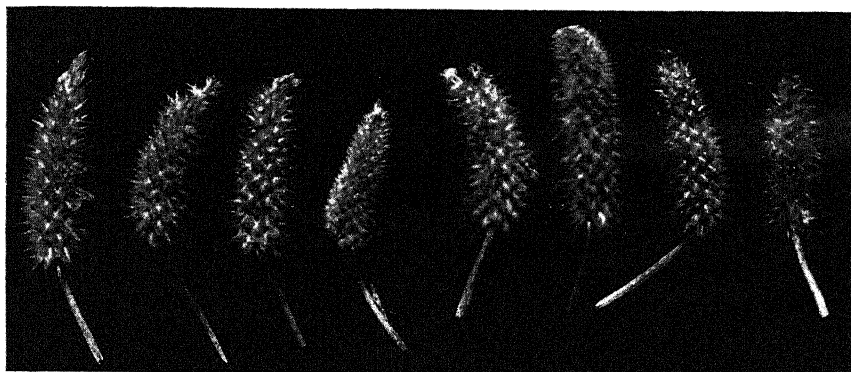
FIG. 2. EFFECT OF BORAX FERTILIZATION ON SEED HEADS OF CRIMSON CLOVER

Left, No borax applied. Note lack of vigor and few matured seeds in apical portion of the head.

Right, 15 pounds borax applied. Heads are vigorous and well filled with seed to apex.

EFFECT OF BORON ON SOYBEANS

Wood's Yellow soybeans were seeded to Norfolk sandy loam in the spring of 1942. The soil was sampled to a depth of 6 inches and is characterized as follows: pH, 5.8; exchange capacity, 2.6, exchangeable Ca, Mg, and K, 0.81, 0.22, and 0.07 m.e./100 gm. soil respectively; organic matter, 1.37 per cent; available P, 55.02 p.p.m.; available boron, 0.25 p.p.m. Treatments consisted of four replications each of a no-borax and a 20-pound borax per acre application broadcast previous to seeding. All plots received a row application of 200 pounds per acre of a 2–12–6 fertilizer.

Borax caused burn on the young seedlings; a reduction in stand of 15.7 per cent, which was significant; and a reduction in height of 14.8 per cent. Borax treatment resulted in a 56 per cent increase in pods on the plants, which was highly significant. The check plots produced an average of 31.14 bushels per acre, whereas the borax-treated plots gave an average of 32.92 bushels per acre. No differences were noted in the germination quality or oil content of the seed.

SUMMARY

Available boron contents of Coastal Plain, Piedmont, and Mountain soils of North Carolina appear to be too low for the most economic production of legume

hays or seeds. The average available boron in the topsoils of each the Coastal Plain, Piedmont, and Mountain regions is 0.24, 0.27, and 0.35 p.p.m. respectively.

The application of borax to soils low in available boron was effective in increasing seed yields of alfalfa, crimson clover, and hairy vetch.

Borax applied to soil at the rate of 5 pounds per acre improved grade quality of peanuts.

The use of borax at a rate of 20 pounds per acre broadcast previous to planting soybeans caused injury, with a reduction in stand and height. The borax, however, gave a highly significant increase in pods per plant, with no difference in yield.

No consistent differences were noted in the germination quality of alfalfa, crimson clover, and soybeans produced at the various boron levels.

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THE USE OF BORAX IN THE LEGUME-LIVESTOCK PROGRAM OF THE SOUTH

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Comprehensive information on the use of borax in the legume-livestock program is not available for many important agricultural areas in the South. The South is by no means a uniform section of the country. The climate, soils, topography, and even the people themselves differ greatly. These and other factors contribute to a variable agriculture. Final conclusions of just how and where the use of borax is important in growing legumes and livestock, accordingly, are not justified.

In Tennessee alone, for example, the Unaka Mountain area has a climatic and growing season similar to that around Chicago. Many different conditions can be seen in the 550 miles across the state to the Mississippi delta cotton-growing country. There are successful livestock farmers in all these areas of major differences, and the most successful of them now follow about the same pattern of legume forage production.

Tennessee conditions have been covered fairly well with test demonstrations, using borax on legumes; and conditions in parts of most of the other Southern States compare with conditions in certain parts of Tennessee. This discussion, therefore, will be confined for the most part to Tennessee, as indicative, at least, of what may be expected elsewhere.

It is a very well known fact that in the last few years the Southern States have been making gradual but significant advancements in agriculture. This has been brought about principally by an increase in forage production, both hay and pasture, and an increase in the numbers of livestock on farms.

To illustrate, the increase in hay production between 1936 and 1942 was 51 per cent in 11 Southern States (excluding Texas and Oklahoma), whereas in the United States as a whole, the increase was only 18 per cent. This has been reflected in the number of forage-consuming animals kept on southern farms, and the trend is increasing the productivity of the land.

During this same period, the dairy and beef cattle numbers increased in the Southern States 15 per cent, while in the United States as a whole, the increase was only 8 per cent. Grain production during this time did not increase significantly in the South. The increase in cattle numbers has been made possible almost entirely by the increase in hay and pasture. The cattle on southern farms have also been fed better, as indicated by the fact that the hay consumed per hay-consuming animal unit has increased 38 per cent, whereas in the United States as a whole, the increase was only 1 per cent. The grain feeding remained virtually the same.

Since the South can not compete successfully with other sections of the country in grain production, it must take advantage of the more favorable long growing

season in the production of forage. To utilize this climatic advantage most successfully, at least two distinct disadvantages must be overcome: first, the naturally infertile and impoverished soil of the major part of the area; and second, the severe drouth normally experienced some time during the summer, the effect of which is naturally much worse on thin land.

To overcome this first disadvantage, the use of limestone and fertilizer has been greatly increased. In Tennessee, for example, the use of limestone from 1936 to 1942 increased over 200 per cent, and the use of fertilizer increased approximately 100 per cent. This increase in lime and fertilizer use is enabling the growth of forage crops which will better withstand the hot, dry periods of summer and the production of pasture crops through the winter months, thus providing grazing intermittently throughout the year.

In Tennessee this more diversified forage production program is essential to safeguard the livestock farmer by assuring adequate nutritious roughage to tide him over unfavorable seasons. Accordingly, livestock farmers have been advised to provide themselves with feed insurance by producing moderate acreages of alfalfa for both hay and pasture, and also as much other winter grazing crops, including crimson clover and small grains, as their land and other facilities will permit, the acreage of these crops, especially alfalfa, has increased materially during the last few years. The use of borax is believed to be partly responsible for this increase and is expected to play a greater part in still further expanding that acreage.

In 1936, a study of 472 farms classified as dairy farms was made to determine their forage production program. At that time, 27 per cent of these dairy farms grew alfalfa, averaging one-fourth acre for each cow on the farm. In 1942, 70 per cent of these same dairy farms grew alfalfa, averaging 0.4 acre per cow. During this period the total milk production in Tennessee has increased 22 per cent, in comparison with 14 per cent increase for the Southern States and 16 per cent for the United States as a whole.

In 1938, it was concluded that the acreage of alfalfa in Tennessee could not continue to be increased because profitable yields were not obtained over a long enough period to justify the cost of establishing a stand. It has now been proved that a major cause of failure was boron deficiency; and that by the use of borax along with limestone and fertilizer, alfalfa can be grown more successfully on the farms that had been growing it, and furthermore, that the acreage can be expanded successfully to farms and to types of soil on which alfalfa could not be grown profitably before borax application entered the Tennessee program of agriculture.

In 1942, reports were obtained of the hay yields from 43 field demonstrations scattered over the state from the Unaka Mountains in east Tennessee to the Mississippi River. These fields were located on what may be termed, for Tennessee, good alfalfa land. The soil had all been limed, and phosphate was added where needed. The addition of 20 pounds of borax per acre increased the yield of hay on an average 26 per cent. Tennessee farmers have never used much potash, but on these demonstrations, where 200 pounds of muriate of potash was used in addition to the borax, the increase in yield was 42 per cent.

A few days ago an inquiry was made of the county agents of Tennessee as to how they thought the use of borax would affect the legume-livestock production in their particular counties. A few of the replies may be cited as typical:

M. L. Alphin, of Hardeman County, a west Tennessee cotton county, bordering the State of Mississippi wrote:

It is a well understood fact that no livestock program can be any better than the feed produced for that livestock. Hardeman County is no exception to the rule that applies to all major cotton counties, and feed has been a very limiting factor in the livestock program. Feed, or the absence of it, can very easily determine the side of the ledger on which the red marks will appear.

The experience of all of our demonstrators has been very good to excellent when borax was applied to alfalfa and red clover. Mr. E. F. Daniel, of the Saulsbury community, says that it means the difference between three tons of alfalfa hay per acre and practically no hay per acre. The experience that he has had has already been directly responsible for three other farmers applying borax to their alfalfa. This same experience has been very evident on all other demonstrations except one. This particular alfalfa demonstration was on real good land and has had almost every available kind of fertilizer applied except borax.

It seems that with the good start we are getting with borax it will mean that we can grow feed for livestock on a par with farmers in the corn belt and others with high producing land. We would like to try borax on pastures to determine if it will help our grasses to stand the summer dry season, and if that should prove effective, I think it would mean more toward improving the livestock program than anything since the land was cleared years ago.

The results of using borax have been most gratifying and I think we have just started in the field of fertilizer research.

Alphin's perspective toward a livestock program based on local feed production appears to be sound.

From the letter received from County Agent Amos in the Upper East Tennessee Valley, it appears that a sounder and more practical livestock program will be developed. The letter, in part, follows:

We have observed with considerable interest the results obtained from demonstrations involving the use of borax on alfalfa and certain other legumes. This practice has, without a doubt, proved to be practical on the soil types common in this county in our legume and livestock program, and there is every indication that such a practice will be common and widespread in the years ahead.

Outstanding results have been noted in the case of alfalfa. The yield of hay has been substantially increased and the general appearance of the stand is greatly improved. This condition reflects itself particularly in lengthening the profitable life of the stand of alfalfa and certainly most farmers will agree that this factor alone is important after taking into consideration the economical production of forage together with the conservation of the soil. Recent hard rains have proved that the land should certainly be kept covered wherever practical.

T. W. Hillsman, county agent in Madison County, reported the beneficial effect of borax when applied on crimson clover. He said that on the gray land of that county, "Crimson clover often 'fires' and finally the plants die. This did not occur when borax was used."

T. L. Mayes, in Franklin County, the largest crimson clover seed producing county in the United States, reported that borax increased the yield of seed, increased the viability, and ripened the crop about three days earlier.

G. L. Cleland, agent in one of the Middle Tennessee bluegrass counties wrote, "It is my experience that borax increases the yield of alfalfa in this county an average of 20 per cent, and in addition, will maintain a stand a year or two longer, which will add another valuable increase. As a result of our demonstration, there is an increased acreage of alfalfa in this county."

Stanley Ezell, agent in Roane County, wrote that, "The use of borax on legumes has proved itself to be a practice that must not be omitted in the Roane County legume-livestock program. Our demonstrations show higher yields in practically every case, ranging from 20 per cent to 80 per cent."

J. Ben Thompson, agent in Cheatham County, said, "I think there is a great possibility in the use of borax on alfalfa and red clover in this county. The results seem to be much greater on thinner soils." This last statement is very significant, for ability to grow alfalfa successfully on thin soils is necessary.

Another very interesting report was received from the county agent in Wayne County, who wrote that, "Two alfalfa demonstrators using borax claimed that the hay from the land treated with borax was eaten better by the livestock to which it was fed." This sounds reasonable. The alfalfa was probably more palatable and nutritious because the leaves were greener and were retained better during the curing process, resulting in leafier, greener hay.

These quotations from the county agents' reports give a general picture of what the agents are thinking. During the last 4 years they have observed a total of over 900 demonstrations where borax has been used on alfalfa, red clover, and crimson clover. Their combined information and conclusions on any subject tested to this extent should be significant.

A number of livestock producers in Tennessee appear to be pointing the way toward more efficient livestock production, using alfalfa, not only as a source of hay, but for pasturing during emergencies when other types of pasture are not available. They have found that alfalfa properly treated, which, of course, includes the use of borax, can be grazed by all types of livestock, if judgment is used with respect to the severity of grazing. One of these farmers, Robert Bell, operates what might now be termed a dairy farm in Dyer County, which is a cotton and livestock county in West Tennessee. He seeded a 12-acre field of alfalfa in the fall of 1940 after he had limed it at the rate of 3 tons an acre. In the spring of 1942, he applied 100 pounds of triple superphosphate, 200 pounds of muriate of potash, and 20 pounds of borax per acre. He cut this alfalfa three times in 1942 with a yield of 1000 bales from the 12 acres, and then pastured it from the time his Korean lespedeza gave out in September until after frost, when crimson clover pasturage became available. An interesting observation was that, with the use of potash and borax as well as limestone and phosphate, he could grow alfalfa at least as successfully on his light buckshot type soil (Grenada) as he could on his well-drained brown soil (Memphis) and that these materials had increased enormously the acreage of his land adapted to alfalfa.

Another dairy farmer, Henry Clark, in East Tennessee, turns dairy cows on his alfalfa in early October and grazes off what would normally be his last cutting. This is grazed until mid-November when he turns his cows on crimson clover

and small grain. This provides pasture until May of the following year, when he turns his animals on bluegrass and white clover, followed by grazing of lespedeza in midsummer. Clark operates only 42 acres of tillable land, and he increased his annual income from $120 in 1932 to over $4000 last year. His principal income is from the 10-cow herd which he is milking.

The importance of borax in legume seed production in the South is covered in another paper of this symposium. Seed in adequate quantities, and at the price which the farmer thinks he is justified in paying, is of great importance in the legume-livestock program.

From experience, it is concluded that borax takes its place as a plant nutrient along with limestone, phosphate, and potash in the development of a legume-livestock program in Tennessee. The extent to which it should be used on crops other than those mentioned remains to be determined by further research and test demonstrations on farms.

EFFECT OF DIFFERENT TYPES OF ORGANIC MATERIALS AND LIME ON SOIL AGGREGATION[1]

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Organic materials such as crop residues, barnyard manure, and cover crops have received considerable attention recently in connection with the conservation of soil and water as well as for increased crop production. Part of the favorable effect of organic matter, whether applied on the surface or incorporated with the soil, may be explained on the basis of improved physical condition of the soil. It is recognized generally that application of organic material increases the infiltration rate, decreases the tendency for soil particles to be dispersed by the beating action of raindrops, thereby reducing the loss through erosion, and improves the air-water relationship in soils. From the large amount of literature on the effect of organic material on soil fertility, it is evident that factors such as soil, climatic condition, microbial population, and type and amount of organic material influence the response that may be expected from the application of organic material under a particular condition. It is logical that these same factors will influence changes in physical properties of the soil, which directly or indirectly affect its susceptibility to erosion. The data presented herein show the effect of widely different organic materials and lime on aggregation in surface and subsurface samples of Gilpin and Holston soils 1, 3, 6, and 12 months after treatment.

MATERIALS AND METHODS

Bulk surface and subsurface samples were collected from Gilpin silty clay loam and Holston clay loam. These soils represent two important series in West Virginia. The Gilpin is an upland soil of shale and sandstone derivation; the Holston is of terrace origin. Certain of the physical and chemical properties of these soils are shown in table 1.

The soils were passed through a 7-mm. screen. A small portion was saved for a check, and the remaining soil received a uniform fertilizer application consisting of 100 pounds per acre each of K_2O and P_2O_5 as muriate of potash and superphosphate, respectively. Half of the soil was limed to approximately pH 6.5 with precipitated $CaCO_3$. Different organic materials, as shown in table 2, were added to the soil at the rate of 12 tons per acre of air-dry material.

Earlier studies (3) indicated that the effect of organic matter on soil aggregation varied at different dates after incorporation of the material with the soil. In the previous studies single pots were used as containers, and the samples for aggregate analysis were taken from the same pots after different periods of decomposition. The mixing and drying required for sampling at different dates materially affected the structural condition of the soil. To study the effects of organic material at different stages of decomposition, without introducing variations due to mixing and drying, the soils were placed in tin cans with a capacity of approximately 1 quart. Eight cans of each treatment were set up, which made

TABLE 1

Some physical and chemical characteristics of the Gilpin and Holston soils studied

SOIL	DEPTH OF SAMPLING	pH	SAND	SILT	CLAY	SPECIFIC GRAVITY	ORGANIC MATTER
	inches		*per cent*	*per cent*	*per cent*		*per cent*
Gilpin.............	0–6	5.28	16.9	54.6	28.5	2.64	1.95
Gilpin.............	7–12	5.00	14.6	53.5	31.9	2.78	0.59
Holston............	0–6	5.09	33.8	43.0	23.2	2.61	2.57
Holston............	7–12	4.65	28.1	39.0	32.9	2.66	0.92

TABLE 2

Organic materials added to soils

ORGANIC MATTER	SOURCE OR STAGE OF MATURITY
Alfalfa...	Hay
Broomsedge...	Mature
Buckwheat straw......................................	Mature
Corn stover...	Mature
Oat straw...	Mature
Peat moss...	German upland
Rye...	Succulent
Rye and vetch...	Succulent
Soybeans (grain, leaves, stems).......................	Hay
Sucrose...	Commercial
Timothy and alfalfa...................................	Hay
Wheat straw...	Mature

it possible to take undisturbed duplicate samples for analysis 1, 3, 6, and 12 months after treatment. Distilled water was added to bring the soils to a moisture content of 20 per cent, a value slightly less than the moisture equivalent. Each can was weighed and covered with wax paper to limit the amount of evaporation. The cans were reweighed at regular intervals and distilled water was added to compensate for that lost by evaporation.

Mechanical analyses were made according to the procedure outlined by Olmsted *et al.* (10). Aggregate distribution was determined by the method described by Yoder (17). Earlier studies indicated that the stability of the aggregates varied, especially in certain soils, with the moisture content of the soil at the time

of analysis. In order to bring all soils to a comparable basis, therefore, all samples were allowed to air-dry before aggregate analyses were made. Percolation rates were determined by the method outlined by Slater and Byers (14). Dispersion ratios were calculated by dividing the >0.05-mm. fraction from the aggregate analysis determination by the >0.05-mm. fraction obtained after dispersion with sodium oxalate. From a limited number of samples studied, this method gave a close approximation to the values obtained with the method described by Middleton (8).
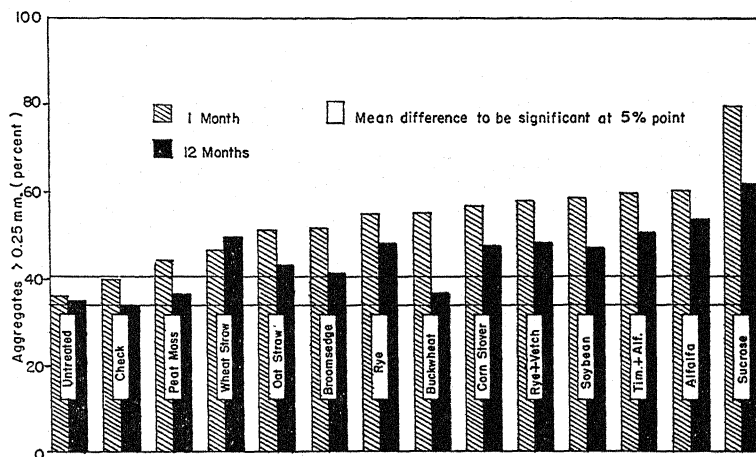
FIG. 1. EFFECT OF DIFFERENT ORGANIC MATERIALS ON THE PERCENTAGE OF AGGREGATES >0.25 MM. IN AN UNLIMED SURFACE SAMPLE OF GILPIN SILTY CLAY LOAM 1 AND 12 MONTHS AFTER TREATMENT

EXPERIMENTAL RESULTS

Effect of different organic materials on aggregation

The effect of different types of organic material on the percentage aggregates >0.25 mm. 1 and 12 months after application for the unlimed surface samples of the Gilpin and Holston soils are presented in figures 1 and 3. Corresponding results for the subsurface samples are shown in figures 2 and 4.

The minimum mean difference required for significance at the 5 per cent point was computed by analysis of variance (15) and is shown graphically in figures 1, 2, 3, and 4. With this as a guide, it is possible to determine whether a significant difference exists between any pair of organic materials. For example, it is to be seen from figure 1 that for the 1-month period the check and peat moss treatments do not differ significantly; all other materials except peat moss are significantly higher than the check, and sucrose is significantly higher than any other treatment. Similar comparisons can be made for all other combinations.

In general, for the 1-month period, soil receiving carbonaceous materials such as oat straw, wheat straw, broomsedge, rye, buckwheat, corn stover, and peat

moss, which are known to be more resistant to decomposition, contained fewer larger-sized aggregates than soil receiving materials with a higher content of nitrogen such as alfalfa, soybeans, rye and vetch, and timothy and alfalfa.
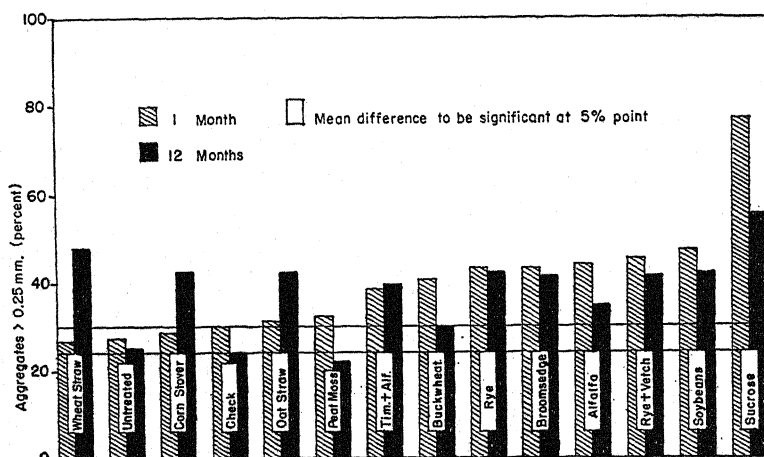
FIG. 2. EFFECT OF DIFFERENT ORGANIC MATERIALS ON THE PERCENTAGE OF AGGREGATES >0.25 MM. IN AN UNLIMED SUBSURFACE SAMPLE OF GILPIN SILTY CLAY LOAM 1 AND 12 MONTHS AFTER TREATMENT

FIG. 3. EFFECT OF DIFFERENT ORGANIC MATERIALS ON THE PERCENTAGE OF AGGREGATES >0.25 MM. IN AN UNLIMED SURFACE SAMPLE OF HOLSTON CLAY LOAM 1 AND 12 MONTHS AFTER TREATMENT

The effect of the different organic materials on aggregation varied with the soil. The Gilpin samples are high in silt and low in organic matter without adequate organic and inorganic colloidal material to bind the smaller particles together into larger aggregates. In soils of this type addition of organic material may be expected to have the maximum effect on aggregation. On the other

hand, there was little change in aggregation regardless of treatment in the Holston surface soil. This sample, taken from an area undisturbed by cultiva-
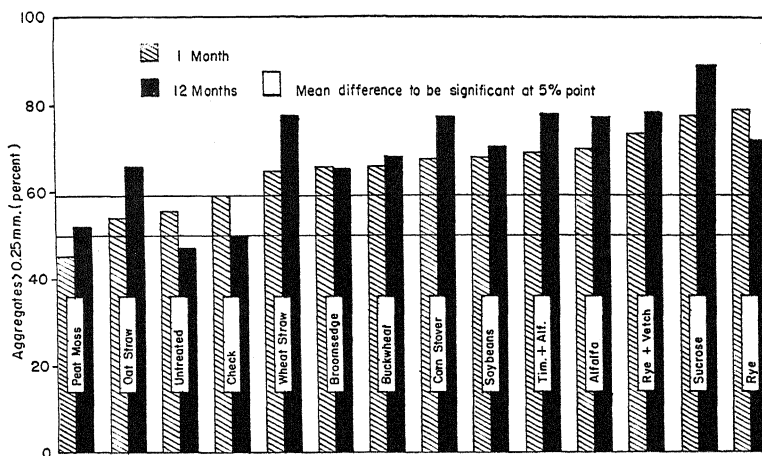
FIG. 4. EFFECT OF DIFFERENT ORGANIC MATERIALS ON THE PERCENTAGE OF AGGREGATES >0.25 MM. IN AN UNLIMED SUBSURFACE SAMPLE OF HOLSTON CLAY LOAM 1 AND 12 MONTHS AFTER TREATMENT

Check

Alfalfa

Sucrose
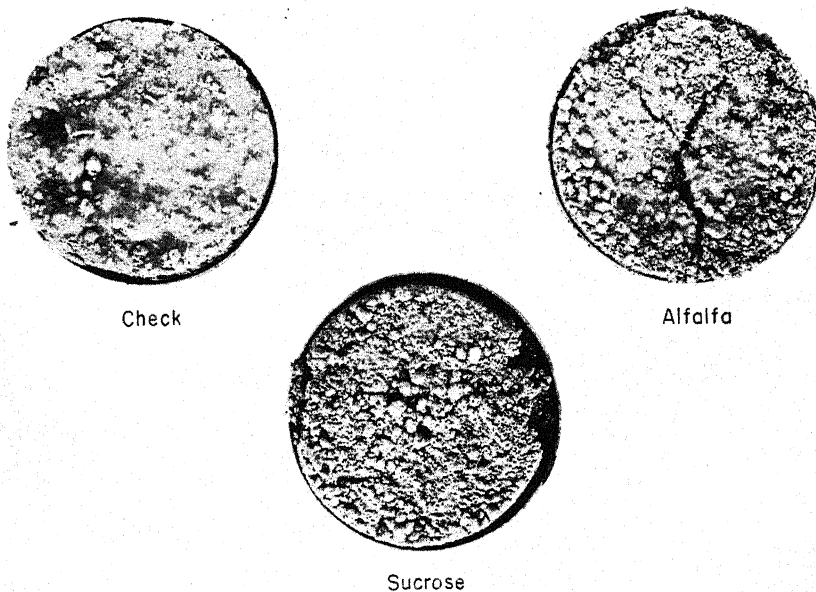
FIG. 5. RELATIVE STRUCTURE IN GILPIN SILTY CLAY LOAM SURFACE SOIL UNDER VARIOUS ORGANIC MATERIALS 6 MONTHS AFTER TREATMENT

tion for many years, is well aggregated, as shown in figure 3. The addition of organic material may be expected to have little if any effect on aggregation in

soils of this type that are well aggregated as the result of past treatment and the presence of adequate binding materials.

The effect of certain materials in maintaining the structure in a condition favorable for rapid penetration of water is shown in figure 5, representing a surface view of the soil as found in the cans after 6 months.   Water had been added from time to time to compensate for that lost by evaporation.   The disturbance caused by pouring water from the beaker dispersed the surface of the check, sealing all large pores and reducing to a minimum the rate at which water enters the soil.   On the other hand, the aggregates in the sucrose- and alfalfa-treated soil maintained their identity throughout the entire incubation period, and water added to the surface entered the soil soon after it was added.
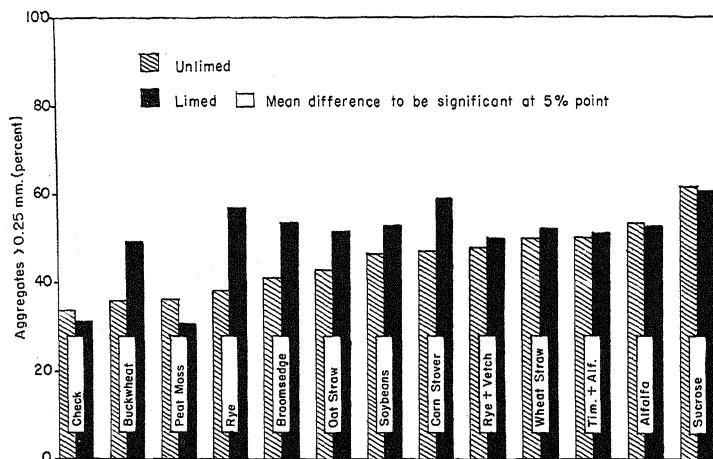
FIG. 6. EFFECT OF LIME ON THE PERCENTAGE OF AGGREGATES >0.25 MM. IN A SURFACE SAMPLE OF GILPIN SILTY CLAY LOAM 12 MONTHS AFTER TREATMENT

Effect of lime on aggregation

The effects of lime on aggregation in the Gilpin surface soil and the Holston subsurface soil are shown in figures 6 and 7, respectively.   These data represent the 12-month sampling period.   Detailed data for the 1-, 3-, and 6-month sampling dates are not shown, because the results are essentially the same as for the 12-month period.   The minimum mean difference required for significance at the 5 per cent point, shown on the graph, makes possible comparisons of the effect of lime on aggregation in the presence of different organic materials. Figure 6 shows that lime caused a significant increase in aggregation when buckwheat, rye, broomsedge, oat straw, soybeans, or corn stover was the source of organic material.   Liming caused no significant change in aggregation for the other materials except peat moss, which showed a decrease.   It is of interest to note that most of the materials, which brought about increased aggregation when lime was added are the ones referred to previously as being more resistant to decomposition.

The effect of lime on aggregation is somewhat different in the Holston sub-surface soil (fig. 7). In this soil liming caused a significant decrease in aggregation with all organic materials except rye, which showed only a small increase.

The effect of lime on aggregation in the Gilpin subsurface soil and the Holston surface soil is not shown, because results, in general, were the same as for the Holston subsurface soil; that is, liming significantly decreased aggregation. These data show that lime alone caused no significant change in aggregation. This is in accordance with previous investigations and data by Baver (1), Bradfield (2), Peele (11), Remisov and Ismaelairch (13), and others. Lime when added with different organic materials had a variable effect upon aggregation, depending upon the soil and the organic material. Although lime alone did not cause a significant change in aggregation, the indirect effect of lime on microbia
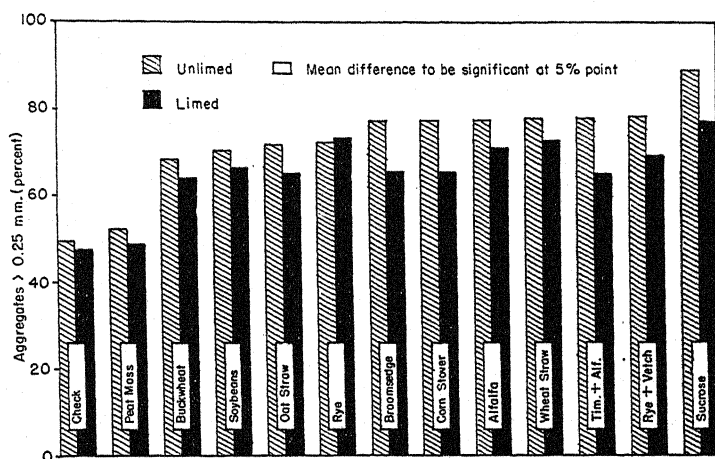
FIG. 7. EFFECT OF LIME ON THE PERCENTAGE OF AGGREGATES >0.25 MM. IN A SUBSURFACE SAMPLE OF HOLSTON CLAY LOAM 12 MONTHS AFTER TREATMENT

activity and plant and root growth is a very important factor in the development and maintenance of a desirable soil structure.

Effect of time on aggregation

The effect of different organic materials on aggregation 1, 3, 6, and 12 months after application was determined for all soils studied. Comparison of the 1- and 12-month periods for the unlimed soils can be made from figures 1, 2, 3, and 4. The percentage of aggregates >0.25 mm. in the unlimed Gilpin subsurface soil 1, 3, 6, and 12 months after the application of different organic material is shown in figure 8.

The data in figure 1 show that in the Gilpin surface soil there is a decrease in aggregation with time for all treatments with the exception of wheat straw, which shows a small increase. The Gilpin subsurface soil reacts somewhat differently, as shown in figure 2. The percentage of aggregates >0.25 mm. was increased

materially by wheat straw, oat straw, and corn stover at the 12-month period as compared to the 1-month period. On the other hand, there was a decrease in aggregation at the 12-month period when buckwheat, alfalfa, soybeans, or sucrose was the source of organic material. It is interesting to note that for the 12-month period, soil treated with wheat straw contains a higher percentage of large-sized aggregates than the soil under any other treatment except sucrose, which in all cases gives a higher percentage than any of the other treatments.

The Holston surface soil (fig. 3), which is well aggregated, shows little change in aggregation with time or with different organic materials. In the Holston subsurface soil (fig. 4), wheat straw, oat straw, corn stover, timothy and alfalfa, alfalfa, sucrose, and peat moss show at the 12-month period significant increases

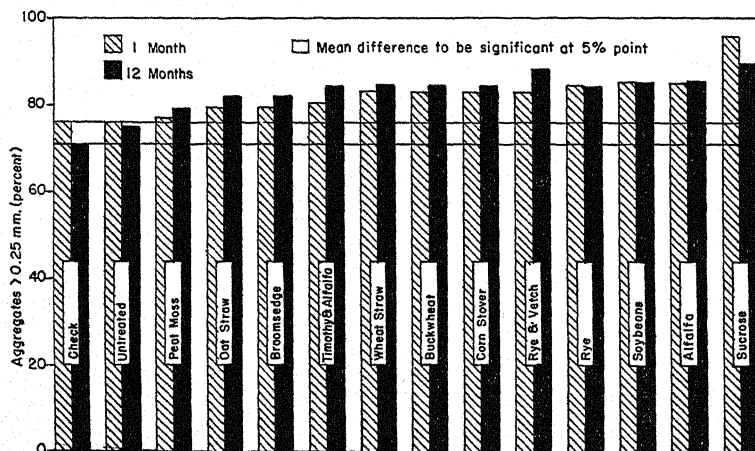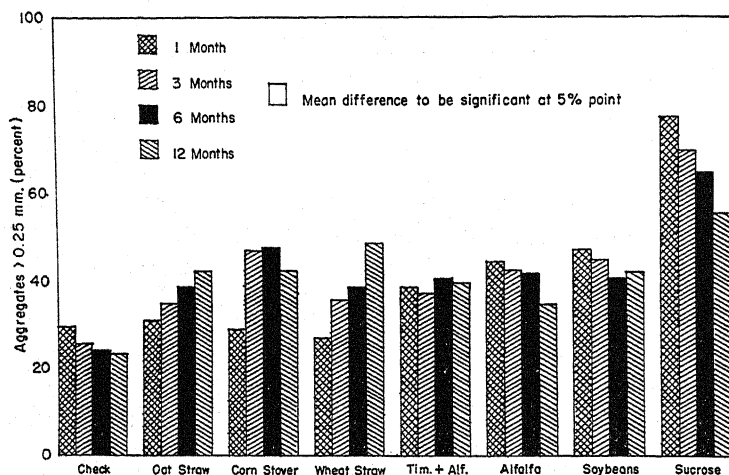FIG. 8. PERCENTAGE OF AGGREGATES >0.25 MM. IN AN UNLIMED SUBSURFACE SAMPLE OF GILPIN SILTY CLAY LOAM 1, 3, 6, AND 12 MONTHS AFTER ORGANIC MATTER APPLICATIONS

in aggregation over the 1-month period, though significant decreases occurred for the check and for rye.

The data in figure 8 show the changes in aggregation in the Gilpin subsurface soil for all sampling dates for certain of the organic materials studied. In general, the changes with time were more or less gradual. Wheat straw, oat straw, and corn stover brought about increased aggregation, whereas alfalfa, soybeans, and sucrose had reached or passed their maximum aggregating effect at the 1-month period and continued to show a decrease with time.

To determine changes in aggregation for incubation periods of less than 1 month, a supplemental experiment was conducted with the Holston surface soil and sucrose. The soil was prepared as described previously, and samples were taken for aggregate analysis 8, 20, and 30 days after treatment. The results are shown in figure 9. Within 8 days the particles >2.0 mm. in size had increased from 14.9 to 32 per cent whereas the fraction >0.25 mm. increased from 56.7 to
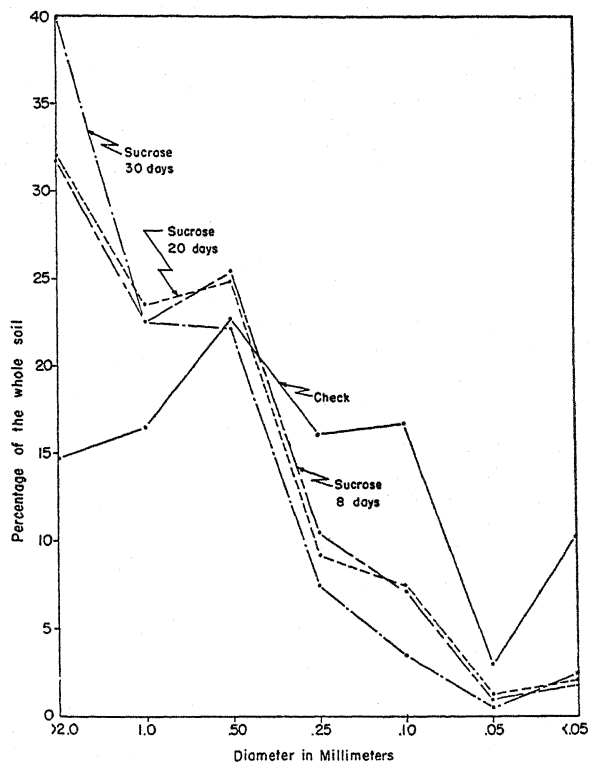
FIG. 9. EFFECT OF ADDED SUCROSE ON THE FORMATION OF AGGREGATES IN HOLSTON CLAY
LOAM SURFACE SOIL AT INTERVALS OF LESS THAN 1 MONTH
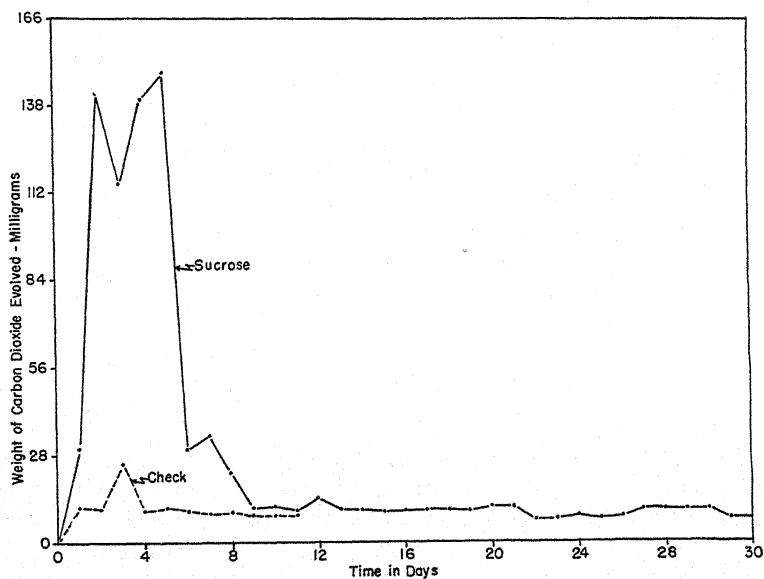
FIG. 10. EFFECT OF SUCROSE ON CARBON DIOXIDE EVOLUTION FROM HOLSTON CLAY LOAM
SURFACE SOIL AT DIFFERENT TIME INTERVALS

99

89.9 per cent.   Little additional change occurred at 20 days, and a small increase in the >2.0 mm. fraction was found 30 days after treatment.

The rate of sucrose decomposition was also studied by determining the CO_2 evolved daily from the check and sucrose treatments.   The data from this study are shown graphically in figure 10.   Carbon dioxide evolution was very rapid from the sucrose treatment at the end of 1 day, reached its peak on the fifth day, and then decreased to approximately that of the check on the ninth day.   The changes in type and number of microflora were not determined.   However, since the rate of CO_2 evolution is related to biological activity, the number and activity of the soil microorganisms must have been materially increased.   It appears,

TABLE 3

Effect of different types of organic matter and lime on the percolation rates of Gilpin silty clay loam and Holston clay loam surface and subsurface soils 1 month after treatment*

Percolation rates in cubic centimeters per hour

TREATMENT	GILPIN SURFACE SOIL		GILPIN SUBSURFACE SOIL		HOLSTON SURFACE SOIL		HOLSTON SUBSURFACE SOIL	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
Check......................	40.3	33.0	55.0	34.0	131.3	134.0	159.0	....
Alfalfa.....................	60.0	52.0	131.0	83.0	792.9	388.0	571.0	442.0
Broomsedge...............	67.0	57.0	116.0	84.0	406.0	248.0	406.0	406.0
Buckwheat................	55.3	....	84.9	....	323.3	....	374.0	....
Corn stover..............	47.8	....	91.9	....	410.8	....	315.0	....
Oat straw................	46.0	48.0	117.0	88.0	356.0	255.0	387.0	317.0
Peat moss................	60.5	....	60.9	....	362.4	....	233.0	....
Rye......................	58.0	62.0	125.0	74.0	421.0	319.0	475.0	303.0
Rye & vetch.............	66.0	....	124.0	....	379.8	....	478.0	....
Soybean hay.............	45.3	....	103.7	....	591.2	....	432.0	....
Sucrose...................	95.0	83.0	108.0	69.0	608.0	362.0	345.0	293.0
Timothy & alfalfa........	51.4	....	112.0	....	341.4	....	495.0	....
Wheat straw.............	65.6	61.0	139.0	94.0	561.0	484.0	504.0	387.0

* Duplicate determinations usually checked within 2 or 3 cc.

therefore, that the time of aggregate formation following the application of the sucrose was very closely related to biological activity.

Percolation rates

In general, the application of the different organic materials increased the number of large-sized aggregates.   Under these conditions there should be more large-sized pores permitting more rapid movement of water through the soil.

Table 3 shows the effect of different organic materials and lime 1 month after application on the percolation rates in the Gilpin and Holston soils.   All materials increased the percolation rate above that for the check, but there was considerable variation among different materials and among soil types.

Lime, with a few exceptions, decreased the percolation rates for the treatment studied.   The increase or decrease in aggregation due to liming, which was discussed above, did not appear to be correlated with percolation rate.   The

percolate from the limed sample was highly colored, indicating the presence of a larger amount of colloidal material in suspension. This is in agreement with data by Myers (9) and others that organic colloidal material is more highly dispersed when saturated with the calcium ion. The dispersed condition of the organic material may have been a factor in decreasing the percolation rates of these laboratory-packed samples. It should be recognized that laboratory percolation rates at best are only an approximation of what may happen under field conditions.

Stability of aggregates

Aggregates formed in a short time by special treatment have been spoken of as pseudo-aggregates and have been considered to have a very limited value in building a stable soil structure. It is generally thought that aggregates to be stable must have developed over a period of years and that the type and stability

TABLE 4

Effect of different organic materials and lime on the stability of aggregates in Gilpin silty clay loam surface soil 1 month after treatment

TREATMENT	UNLIMED			LIMED		
	Aggregates > 0.25 mm.		Stable aggregates	Aggregates > 0.25 mm.		Stable aggregates
	Before shaking	After shaking 2.0 min.		Before shaking	After shaking 2.0 min.	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Check......................	30.7	13.9	45.2	30.2	13.0	43.1
Alfalfa.....................	55.1	36.1	65.5	51.8	25.0	48.2
Peat moss..................	39.5	12.2	35.4	37.7	12.1	32.1
Sucrose....................	74.4	47.2	63.5	74.0	44.8	60.6
Wheat straw...............	41.4	20.4	49.4	48.9	25.3	51.7

of the aggregates formed depend upon the climatic conditions, the vegetative cover, and the physical and chemical properties of the soil. To obtain information on the stability of the aggregates in this study a 25-gm. sample of soil and 100 cc. of distilled water were placed in a 500-cc. Erlenmeyer flask and shaken for 2 minutes at 25 r.p.m. in an end-over-end shaker before fractionation in the usual manner. Aggregates resistant to this treatment, which is purely arbitrary, were considered as stable. The percentages of stable aggregates in limed and unlimed Gilpin surface soil 1 month after treatment with certain organic materials are shown in table 4.

From these data it is evident that the aggregates formed in the alfalfa and sucrose treatments in the unlimed soil were stable under the conditions of this experiment. Wheat straw increased the stability of the aggregates formed above that of the check, although the difference may not be significant; peat moss decreased the percentage of stable aggregates. The use of lime in addition to organic matter decreased the stability of the aggregates formed as compared to organic matter alone.

The data in table 5 show the stability of aggregates in Carrington silt loam from Minnesota, and Aiken clay loam from Oregon. The aggregates in these soils have developed over a long time under natural conditions. The Aiken soil has 63.9 per cent and the Carrington 33.0 per cent stable aggregates >0.25 mm. in diameter after shaking for 2 minutes in an end-over-end shaker. The alfalfa and sucrose treatments (table 4) resulted in 65.5 and 63.5 per cent stable aggregates respectively. On the basis of these data, therefore, aggregates formed during a short time are stable. However, if no additional treatment were given and the soils were all subjected to the same conditions for a period of years, it is probable the initial aggregating effect of the organic matter treatment would

TABLE 5

Stability of aggregates in Carrington silt loam and in Aiken clay loam

SOIL	AGGREGATES > 0.25 MM.		STABLE AGGREGATES
	Before shaking	After shaking 2 minutes	
	per cent	*per cent*	*per cent*
Aiken clay loam.....................	42.3	27.0	63.9
Carrington silt loam................	56.2	18.5	33.0

TABLE 6

Correlation coefficients of aggregation and dispersion ratio in Gilpin silty clay loam and Holston clay loam surface and subsurface soils

CHARACTERISTICS CONSIDERED	CORRELATION COEFFICIENT*			
	Gilpin		Holston	
	Surface	Subsurface	Surface	Subsurface
Aggregates > 0.25 mm. and dispersion ratio............................	−.93	−.85	−.62	−.89
Aggregates > 1.0 mm. and dispersion ratio............................	−.71	−.62	−.52	−.66

* Value of r at the 1 per cent point is 0.25.

disappear, whereas the aggregates in the Carrington and Aiken soils would be less subject to change. The wide difference in the stability of the aggregates in the Carrington and Aiken soils is further evidence that aggregate analysis by a given procedure may fail to characterize the structural condition of soils in relation to their susceptibility to erosion.

Relation of aggregation to the dispersion ratio

Dispersion and aggregation should be closely related. A change in dispersion ratio may not necessarily give a corresponding change in aggregates >0.25 mm.; however, since the finer soil particles which form aggregates as the result of a particular treatment do not necessarily distribute themselves uniformly throughout the size range of particles. The data collected in this study include a wide
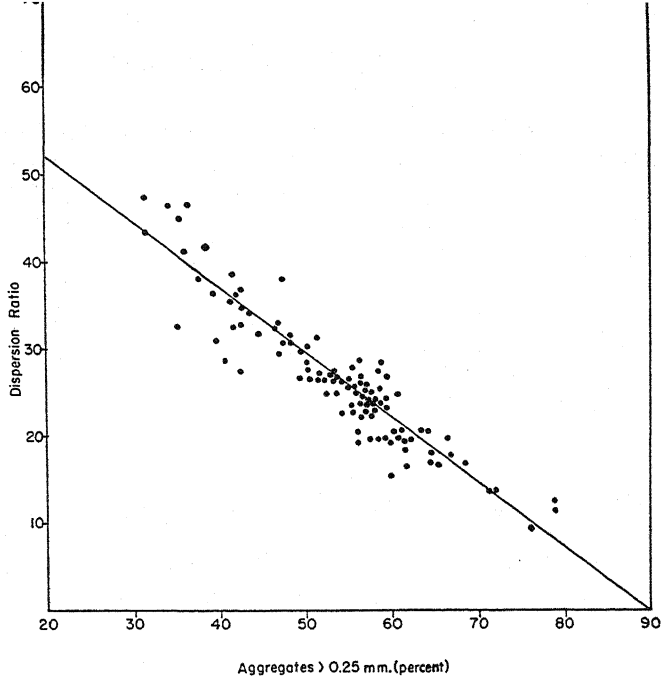
FIG. 11. RELATION OF DISPERSION RATIO TO AGGREGATES >0.25 MM. IN GILPIN SILTY CLAY
LOAM SURFACE SOIL TREATED WITH 13 ORGANIC MATERIALS, UNLIMED
AND LIMED, 1, 3, 6, AND 12 MONTHS AFTER TREATMENT
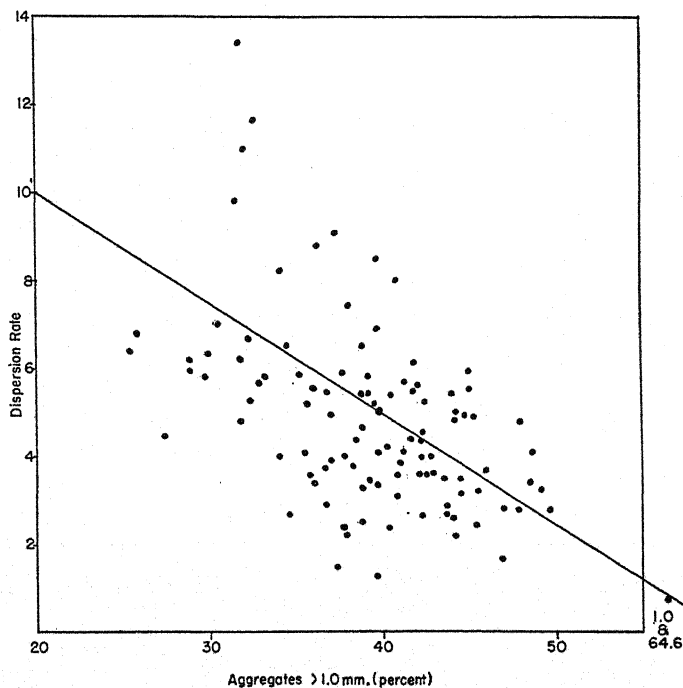
FIG. 12. RELATION OF DISPERSION RATIO TO AGGREGATES >1.0 MM. IN HOLSTON CLAY
LOAM SURFACE SOIL TREATED WITH VARIOUS ORGANIC MATERIALS, LIMED
AND UNLIMED, 1, 3, 6, AND 12 MONTHS AFTER TREATMENT

range in treatment and offer an opportunity to compare dispersion ratio and aggregation as criteria of effectiveness of organic matter treatments.

Simple correlation coefficients between dispersion ratio and aggregates >0.25 mm. and aggregates >1.0 mm. are presented in table 6. The negative correlation between these variates is highly significant.

The correlations between aggregates >1.0 mm. and the dispersion ratio are not so high as similar figures for the relation between aggregates >0.25 mm. and the dispersion ratio. The failure of the two methods to give the same general picture can be more readily seen from the scatter diagrams, figures 11 and 12, representing respectively the highest and the lowest relationship found. For the Gilpin surface soil the same general conclusion would have been reached by grouping aggregates >0.25 mm. or by using the dispersion ratio. Comparing aggregates >1.0 mm. and the dispersion ratio in the Holston surface soil reveals that the effect of certain treatments would be considerably different when measured by the two methods. It is not known which of the two more nearly characterizes soil structure as related to erodibility. Possibly one of the many other methods of grouping used by other investigators would be more satisfactory. Unless definite evidence is available to show the need for the different grouping it would aid materially in interpreting aggregate analysis data to have all results reported on some uniform basis.

DISCUSSION

Soil type and past management materially influence changes in aggregation resulting from a single application of organic material. It has been shown previously (3) that soils containing a high percentage of an active inorganic colloidal material or of organic matter have more cementing material present, and the effect of additional organic matter in improving the physical properties of the soil is minimized. Sandy soils are a somewhat different problem. The amount of silt and clay in proportion to the sand is often too small to produce other than a single-grain structure. The application of organic matter under this condition will have little, if any, effect upon aggregation of the soil. It is not definitely known what proportions of sand, silt, and clay are necessary for organic matter to show changes in aggregate distribution. This will vary not only with percentages of the different fractions present, but also with the size and type of the different fractions. Studies of the effect of cover crops on soils containing varying amounts of sand show that little, if any, effect can be expected in sandy soil with a silt and clay content almost high enough to class the soil as a silt loam. In view of the extremely rapid changes in aggregation and the tendency for the effect to disappear with time, however, it is possible that in these studies the aggregating effect had already disappeared. The amount of material turned under with a cover crop is generally much less than was applied in this study, and the changes in aggregation are relatively small for the low rates of organic materials (4).

It is significant from the standpoint of water relationships and erosion control that different organic materials vary in their effect upon soil aggregation. The

materials that are resistant to decomposition will have little, if any, effect upon the size distribution of the soil aggregates but will be effective over a longer period in an actual mechanical loosening of the soil. The materials that decompose rapidly will bring about rapid changes in the soil properties which should be instrumental in decreasing erosion. This may be of value in certain instances in connection with turning under cover crops, with barnyard manure, or with various methods of handling crop residues. The use of these materials would be especially helpful if the land is planted to corn or some other intertilled crop that leaves the soil unprotected during the time of year when a large number of intense storms occur.

It is evident that the initial rapid change in aggregation resulting from a single application of organic materials is directly or indirectly related to biological activity. The improved physical condition is probably the combined effect of the by-products of decomposition, whether transitory or stable, and the large amount of cells and materials synthesized by the microorganisms. This is in accordance with data by Peele (12) which show that sucrose added to a sterile soil without inoculation did not affect aggregation, but when the soil was inoculated with a bacterial culture the percentage of water-stable aggregates increased rapidly. He found that the stability of the aggregates seemed to vary with the viscosity of the mucus produced by the various organisms, the most viscous mucus producing the most stable aggregates. Also of interest in connection with the different types of organic material used in the study is the fact that not all organisms affect the soil to the same degree (6, 7, 16) and that certain organisms are present in large numbers during stages when the different materials of decomposition are present.

Recent studies (5) on the physicochemical combinations of organic materials and clay minerals found commonly in soils may be helpful in explaining the differences found in the amount and stability of aggregates formed following the application of organic materials of different chemical composition to soils containing clay minerals that differ in their physical and chemical properties.

SUMMARY

The effect of a single application of different organic materials and lime on aggregation in surface and subsurface samples of Gilpin silty clay loam and Holston clay loam may be summarized briefly as follows:

Organic materials that decompose rapidly increase aggregation within a few days after they are incorporated with the soil, have their maximum effect in about 20 to 30 days, and then gradually lose their effectiveness with time. The materials that are slower to decompose require a longer time to exert their binding effect but continue to be effective over a longer period. Materials that are relatively inert have little, if any, effect upon aggregation.

Lime when added with the different organic materials significantly decreased aggregation in the subsurface samples of the Gilpin and Holston soils and in the Holston surface soil. In the Gilpin surface soil lime significantly increased aggregation when added with buckwheat, rye, broomsedge, oat straw, soybeans, or corn stover; decreased aggregation with peat moss; and did not cause a significant change in the other organic materials studied.

The percolation rate of laboratory-packed samples appears to be affected both by the mechanical loosening of the soil and the aggregating effect of the organic material. In general, lime decreased the percolation rate. The organic colloidal material was more highly dispersed in the presence of lime, which may have been a factor in decreasing the percolation rate.

Aggregates formed in a short time from a single application of organic material were relatively stable under the conditions of this study. The dynamic nature of aggregation in soils necessitates carefully controlled conditions if results are to be comparable between and within seasons.

The relation of aggregate distribution to the dispersion ratio varies with different soils and with the type of organic material. Correlation coefficients between different grouping of aggregates and the dispersion ratio are shown.

In general the addition of organic material to these soils improved some of the physical characteristics which are recognized as affecting the susceptibility of a soil to erosion.

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NORMAL SEASONAL CHANGES OF OXYGEN AND CARBON DIOXIDE PERCENTAGES IN GAS FROM THE LARGER PORES OF THREE ORCHARD SUBSOILS

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Since 1938 a study has been under way of the seasonal changes of oxygen and carbon dioxide percentages in gas taken from orchard subsoils. Several papers have been written on the work as it has gone along (3, 4, 5, 10). This article is concerned with the "average" seasonal changes of oxygen and carbon dioxide percentages in three soil types at different distances from the soil surface, and with the variations from average occurring in the years studied thus far.

SAMPLING LOCATIONS

Two permanent gas sampling stations were located 3 feet from the trunks of bearing McIntosh apple trees growing on each of three soil variations in the Cornell University orchard, Ithaca, N. Y. The soils[1] are a sandy loam, a light silty clay loam, and a silty clay.

The sandy loam soil is rather uniform in texture and structure to a depth below 6 feet. The field moisture capacity of the subsoil layer is about 22 per cent of volume, and total porosity is about 38 per cent of volume, the 16 per cent difference representing the macropore space.

The light silty clay loam soil is also rather uniform in texture and structure to a depth below 6 feet. The field moisture capacity of the subsoil layer varies between about 36 and 38 per cent of volume, total pore space varies between about 41 and 45 per cent, with about 6 to 8 per cent of volume as macropore space.

The silty clay soil is heavier in texture in the upper 3 feet of the profile and is more compact at a depth of 4 feet and below than is the light silty clay loam soil. Because of the heavier texture and greater compaction, the soil volume represented by macropore space decreases from about 6 per cent at a depth of 1 foot to 1 per cent or less below 3 feet.

The topography at all locations is gently rolling. The soil surface immediately above the sampling wells has been kept clear of vegetation. Quack grass covered the surface of the soil adjacent to the sampling locations.

METHODS OF SAMPLING AND ANALYSIS

Soil air samples were taken under partial vacuum from wells that had been placed previously at the desired depths. A sampling well consists of a piece of 20-mm. glass or copper tubing 75 mm. long, filled with glass wool, open at the bottom and closed at the top with a one-hole rubber stopper. Inserted in the

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[1] These soils are described more fully in an earlier publication (5).

stopper and connecting the inside of the well with the surface of the ground is a ⅛-inch copper tube. This tube is sealed into the ground above the well with pulverized subsoil that is compacted into a dense plug.

A mercury pump is used to draw the gas samples into 35-cc. glass-stoppered bottles, and the samples are analyzed by the Haldane method (9). On the basis of studies of the method (3), the following routine procedure has been adopted: 150 cc. of gas is withdrawn; then two 35-cc. bottles are filled with gas consecutively. These constitute the duplicate samples taken from a well.

TABLE 1

Monthly precipitation at the United States Weather Bureau, Ithaca, New York

In inches

	1938	1939	1940	1941	1942	1938–1942 AV.	74-YEAR AV.
January............	1.72	2.82	0.72	1.46	1.30	1.60	2.08
February..........	2.16	3.21	3.25	1.05	1.90	2.31	1.87
March.............	1.12	2.82	3.47	1.87	4.90	2.84	2.35
1st quarter.......	5.00	8.85	7.44	4.38	8.10	6.75	6.30
April.............	2.52	2.51	3.59	2.68	1.53	2.57	2.48
May..............	2.43	1.84	5.35	1.59	3.31	2.90	3.38
June.............	2.25	2.41	4.37	3.24	2.39	2.93	3.63
2nd quarter.......	7.20	6.76	13.31	7.51	7.23	8.40	9.49
July.............	2.31	3.17	2.87	6.42	5.54	4.06	3.48
August...........	3.59	1.46	2.73	1.57	4.68	2.81	3.21
September.........	6.71	2.86	2.79	1.03	3.54	3.38	3.03
3rd quarter.......	12.61	7.49	8.39	9.02	13.76	10.25	9.72
October...........	0.59	2.49	1.87	1.73	2.90	1.92	2.91
November.........	2.22	0.55	1.73	1.78	3.02	1.86	2.40
December..........	2.42	2.76	2.44	2.25	5.56	3.09	2.20
4th quarter.......	5.23	5.80	6.04	5.76	11.48	6.87	7.51
Year............	30.04	28.90	35.18	26.67	40.57	32.27	33.02

Analysis is completed, with few exceptions, within 24 hours of the time of sampling.

PRECIPITATION

Precipitation at the Ithaca Weather Bureau for the period under study is summarized by months and quarters in table 1. In the 5-year period, 1938–1942, average annual rainfall was 32.27; this was 0.75 inch below the 74-year average at Ithaca. In 1938, 1939, and 1941, annual rainfall was below normal. In 1940, rainfall was 2 inches above normal; and in 1942, there was an excess of

TABLE 2

Monthly mean air temperatures at the United States Weather Bureau, Ithaca, New York
In degrees F.

	1938	1939	1940	1941	1942	1938–1942 AV.
January...................	25.2	26.4	15.3	24.0	25.0	23.2
February..................	28.9	28.8	22.8	23.6	21.4	25.1
March....................	40.2	32.2	26.3	27.6	37.3	32.7
April.....................	48.2	43.5	40.7	51.6	50.5	46.9
May......................	56.2	60.8	56.4	58.0	60.2	58.3
June.....................	66.4	67.0	65.4	68.2	67.0	66.8
July.....................	72.5	71.8	70.4	72.6	70.6	71.6
August...................	73.2	73.6	67.2	66.8	67.6	69.7
September................	57.8	64.0	59.6	64.3	61.3	61.4
October..................	53.2	51.2	47.3	53.2	53.2	51.6
November................	41.3	36.8	38.8	43.6	40.0	40.1
December................	30.4	30.2	32.5	32.5	25.0	30.1

TABLE 3

Soil temperatures* at different depths under quack grass cover in the Cornell University Orchard, Ithaca, New York, 1941–1942
In degrees F.

	1-FOOT DEPTH	2-FOOT DEPTH	3-FOOT DEPTH	4-FOOT DEPTH	5-FOOT DEPTH	6-FOOT DEPTH
1941						
April 21......................	53	48	44	42	41	40
May 6.......................	51	48	46	44	43	42
May 27......................	57	52	50	48	46	45
June 12.....................	59	57	56	54	52	51
June 30.....................	69	64	60	57	54	53
August 9....................	64	61	60	59	57	54
September 5.................	66	63	61	60	59	58
October 7...................	63	60	58	57	56	55
November 27................	44	46	48	50	52	52
1942						
January 23..................	40	41	43	45	46	48
March 19...................	42	43	44	45	46	47
March 27...................	43	44	44	45	47	46
April 29....................	54	51	50	48	47	46
May 26.....................	53	53	52	51	49	49
June 15....................	59	59	57	54	51	50
June 26....................	59	58	56	55	54	52
August 19..................	64	62	60	59	57	55
September 16...............	64	61	60	59	58	56
October 12.................	53	55	56	55	55	55
November 9................	46	49	51	52	53	54

* Each figure represents the mean of temperature readings on six thermocouples, two of which were in the sandy loam soil, two in the light silty clay loam soil, and two in the silty clay soil.

more than 7 inches over the long-time average.   In the 5-year period, there were four quarters during which precipitation was more than 10 inches: the third quarter of 1938, the second quarter of 1940, and the third and fourth quarters of 1942.   Rainfall during July, 1941, amounted to 6.42 inches.

AIR AND SOIL TEMPERATURE

Monthly mean air temperatures at the Ithaca Weather Bureau for the 1938–1942 period are recorded in table 2.

TABLE 4

Fluctuations of ground water during the early growing season at the gas sampling locations, Cornell University Orchard, Ithaca, New York, 1938–1942*

MONTH	APRIL		MAY				JUNE				JULY			
Week	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
	Distance of ground water from soil surface (inches)													
1938														
Light silty clay loam...	7	24	32	38	24	41	†	†	†	†	†	†	†	†
Silty clay........	2	10	14	22	8	19	27	†	†	†	†	†	†	†
1939														
Light silty clay loam...	‡	9	28	40	†	†	†	†	†	†	†	†	†	†
Silty clay........	‡	6	22	30	44	†	†	†	†	†	†	†	†	†
1940														
Light silty clay loam...	26	15	13	28	‡	20	13	34	†	†	32	44	†	†
Silty clay........	14	15	13	18	18	18	17	26	40	43	21	32	39	†
1941														
Light silty clay loam...	13	31	‡	37	†	†	†	†	†	†	†	†	†	†
Silty clay........	16	20	‡	22	‡	37	39	‡	‡	39	†	†	†	†
1942														
Light silty clay loam...	24	‡	43	‡	‡	27	‡	†	‡	†	‡	†	‡	†
Silty clay........	14	‡	23	‡	‡	‡	‡	35	‡	40	‡	†	‡	†

* Each figure is the average of two determinations at a distance of 10 feet from the experimental trees.   The measurements were made in open-sided cylinders of light sheet iron 1 inch in diameter and 4 feet long, placed in holes made by a California soil tube (11).
† No water in the ground-water tube.
‡ No measurements made.

No records of soil temperature were taken prior to 1941.   The seasonal changes of soil temperature at the different sampling depths in 1941 and 1942 are indicated in table 3.

GROUND WATER AND SOIL MOISTURE

The seasonal decrease in accumulated gravitational water in the pores of the two heavier soil profiles is indicated in table 4.   There was no "ground water" at any time in the sandy loam subsoil at the depths sampled.

Unfortunately it was impossible to measure quantitatively the seasonal drying of these soil profiles at the sampling stations without risking a change of the experimental conditions. However, the seasonal trend of soil moisture in 1942 is indicated in table 5 in the resistance readings on Bouyoucos gypsum blocks (1) inserted at the gas sampling depths under apple trees adjacent to the ones under experiment on the two heavier soil profiles. As the large single-grained

TABLE 5

Resistance readings* on Bouyoucos soil moisture blocks set in two orchard soils at different depths, 1942

Log R in ohms

DATE	SILTY CLAY			LIGHT SILTY CLAY LOAM		
	1-foot depth	3-foot depth	5-foot depth	1-foot depth	3-foot depth	5-foot depth
Jan. 23	3.10	3.01	3.72	3.63	3.80	3.86
Mar. 19	2.91	2.71	2.66	2.95	2.79	2.74
Mar. 27	2.95	2.65	2.63	2.96	2.76	2.70
Apr. 29	3.08	2.57	2.57	2.91	2.65	2.62
May 26	2.95	2.54	2.44	2.90	2.69	2.60
June 15	4.60	2.90	2.54	4.35	3.77	2.83
June 26	4.78	3.42	2.72	4.68	4.56	3.33
July 23	....	....	....	4.40	4.73	4.24
Aug. 19	4.36	2.65	2.70	3.74	4.52	4.21
Sept. 16	4.60	3.63	2.99	4.94	4.99	4.57
Oct. 12	4.63	3.93	3.07	5.08	4.75	4.58
Nov. 19	3.54	2.70	2.57	4.37	4.20	3.72

* Each figure represents the mean of resistance readings on two gypsum blocks which were fitted tightly into holes cut into the face of a ditch. The ditch was filled after the blocks were set. Edlefsen, Anderson and Marcum (7) in careful calibrations under field conditions found that at field moisture capacity the electrical resistance of these blocks buried in soils of widely different textures was about 500 ohms, and the resistance of blocks buried in soil at the permanent wilting percentage was about 500,000 ohms with most of the available water exhausted from the soil when block resistance was 10,000 ohms.

particles of the sandy loam subsoils did not furnish satisfactory capillary connection with the gypsum blocks, no data are presented for that soil.

AVERAGE SEASONAL CHANGES OF OXYGEN AND CARBON DIOXIDE PERCENTAGES
IN SOIL GAS SAMPLES

Graphs of the average seasonal changes of oxygen and carbon dioxide percentages of soil gas sampled at 1-, 3-, and 5-foot depths in the three soils during the 1938–1942 period are presented in figure 1. Each point on these graphs is the mean of the oxygen or carbon dioxide analyses of gas samples taken during a 1-month period throughout the 5 years. Since the dates of sampling varied from year to year, a single point represents the average of 6 to 20 analyses. In general, the points for the period from April to November represent averages of
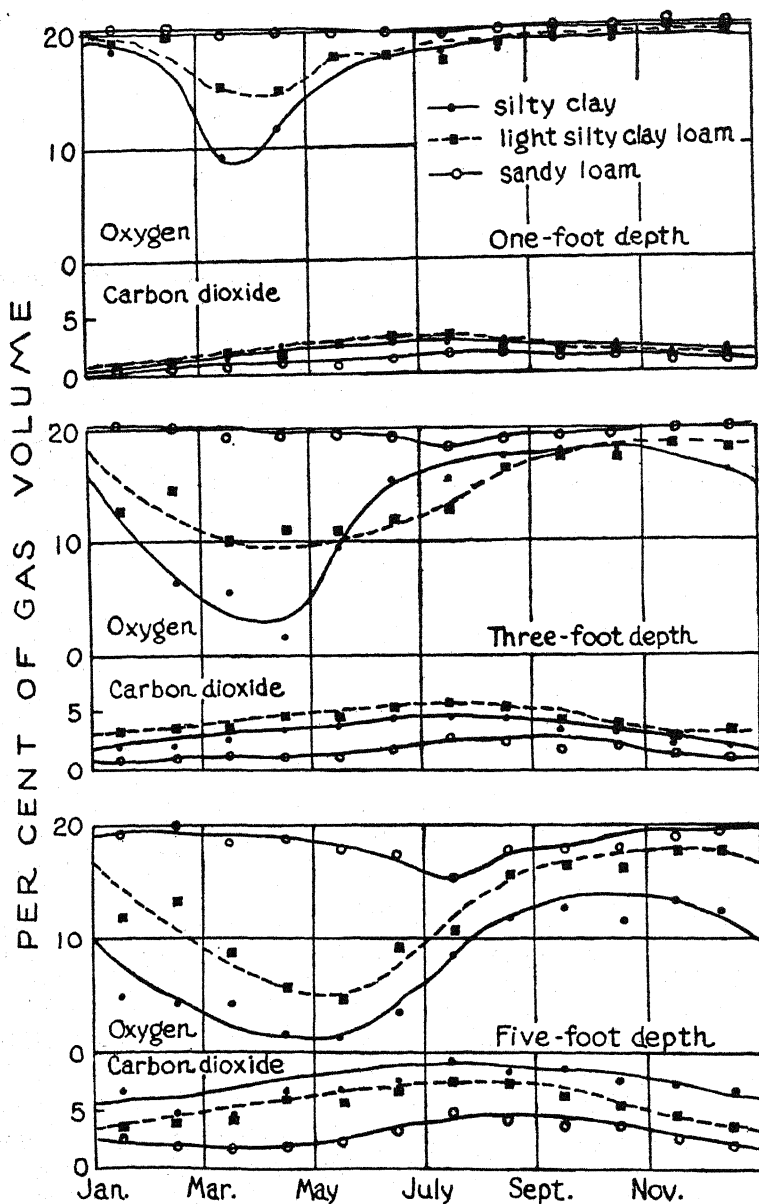
FIG. 1. NORMAL SEASONAL CHANGES OF OXYGEN AND CARBON DIOXIDE PERCENTAGES IN
GAS FROM DIFFERENT DEPTHS IN A SILTY CLAY, A LIGHT SILTY CLAY LOAM,
AND A SANDY LOAM SUBSOIL

12 to 20 analyses, and the points for the remainder of the year represent averages
of 6 to 12 analyses.

In determining a mean oxygen percentage for a 1-month period (fig. 1),
oxygen percentage was recorded as zero for dates when no gas sample could be

obtained from a well.   In the case of the mean carbon dioxide level, the averages
are based on actual carbon dioxide analyses only.

SEASONAL CHANGES OF OXYGEN PERCENTAGE IN SOIL GAS SAMPLES DURING THE 5-YEAR PERIOD

Figure 2 shows the changes of oxygen percentage in gas samples taken at 1-,
3-, and 5-foot depths in the three soils, and the cumulative annual rainfall for
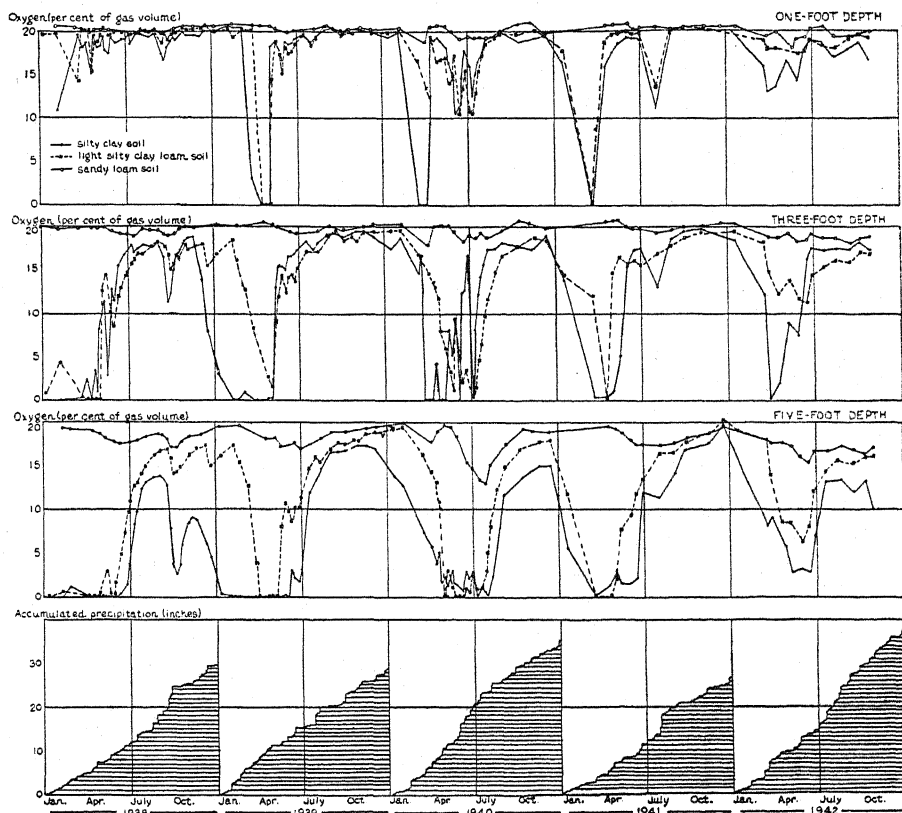
FIG. 2. ACCUMULATED PRECIPITATION AND SEASONAL CHANGES OF OXYGEN PERCENTAGE IN
A SILTY CLAY, A LIGHT SILTY CLAY LOAM, AND A SANDY LOAM
SUBSOIL DURING A 5-YEAR PERIOD, 1938–1942

each of the 5 years under study.   Each point on the oxygen curves represents
the average of the determinations from two gas sampling stations.

RESULTS

Normal seasonal trends

At a depth of 5 feet in the silty clay subsoil, oxygen was restricted to a quarter
or less of the partial pressure found in normal air during the period from about
February 1 to July 1 (see figure 1).   In the period from September to December,

oxygen reached a maximum percentage three quarters of that found in normal air. The same trend occurred in the light silty clay loam profile at that depth, but the mean oxygen percentages were consistently higher. In the sandy loam soil at the 5-foot depth, the oxygen percentage remained higher throughout the year than in either of the other soils, and the minimum, 15 per cent of volume, was not reached until midsummer.

At the 3-foot and 1-foot depths, the oxygen percentage in the two heavier soils followed the same general pattern as at the 5-foot depth. Minimum values occurred during late winter and early spring; maximum values, in late summer and autumn. But the period of restricted oxygen percentage was shorter, and the minimum levels were higher at the 3-foot depth than at the 5-foot depth, and at the 1-foot depth than at the 3-foot depth.

In the sandy loam subsoil the oxygen percentage was only slightly below that of air at the 3-foot and 1-foot depths. At these depths, as at the 5-foot depth in the sandy loam soil, the oxygen curves dipped to minimum levels in midsummer instead of in early spring.

Carbon dioxide percentage was higher in gas from the heavier subsoils than in gas from the sandy loam subsoil and increased with depth. The fluctuation during the growing season was much less than in the case of oxygen percentage. For a given soil and depth, the maximum percentages seemed to occur during midsummer.

Reasons for normal seasonal changes in gas composition

In the heavier profiles the lowest oxygen levels occurred when soil moisture was highest, and often coincided with the time when gravitational water was present (fig. 1 and tables 4 and 5). Soil temperature was close to the minimum for the year during this period (table 3). Thus it seems probable that in the heavier profiles the minimum oxygen levels were primarily caused by restricted opportunity for gaseous diffusion, despite temperatures that were relatively unfavorable for biological activity.

In the sandy loam subsoil, the normal minimum oxygen level seemed to occur in midsummer, when soil moisture was relatively low but when soil temperature was close to maximum. This seems to indicate that with opportunity for diffusion relatively favorable throughout the year, temperature was the most important factor controlling the oxygen percentage of the subsoil atmosphere. That is, the rate of biological activity in the subsoil was great enough during the period of highest temperatures to depress the oxygen level of the subsoil air a little.

Carbon dioxide percentages (fig. 1) varied less during the growing season than did the oxygen percentage. In all three soils at the three different depths, average carbon dioxide percentage reached a maximum in midsummer, when soil temperature was high. This was presumably due to the relatively high rate of biological activity in midsummer. The solubility of CO_2 in water and, in some layers, the formation of calcium bicarbonate when carbon dioxide was present doubtless removed from the soil gas a larger proportion of the carbon dioxide

evolved in respiration during the winter months when the rate of activity was low than in the summer months when the rate was higher.

Variations from normal in seasonal trends of oxygen percentage

Figure 2 gives a continuous chart of the seasonal fluctuations of oxygen percentage at 1-, 3-, and 5-foot depths in these soils for the period 1938–1942. The normal seasonal trends, shown in figure 1, are evident in all of the 5 years. The figure also shows the differences in the oxygen curves for the different years—differences that seemed to be mainly associated with rainfall.

In 1939 and again in 1941, precipitation was considerably below normal. In 1940 and in 1942, precipitation was above normal. The percentages of oxygen in gas samples at the 5- and 3-foot depths rose from the minimum levels earlier in the spring, reached higher maximum levels, and remained relatively high longer in 1939 and 1941 than in the years of greater rainfall.

Rainfall for one month exceeded 6 inches in September, 1938, and in July, 1941. In both instances the rainfall for the previous month was more than 3 inches, and the rainfall for the month following was very low. Under those conditions, there appeared to be a marked temporary depressing effect of the heavy precipitation on the subsoil oxygen percentage.

Rainfall for a 2-month period in the growing season approached 10 inches in May and June, 1940, and in July and August, 1942. In 1940 the time of minimum oxygen level for the heavy soils was prolonged by the above-normal rainfall; in 1942 the heavy rainfall stopped the rise in oxygen percentage in gas from the heavier subsoils, and in some cases caused a slight temporary drop in oxygen percentage.

DISCUSSION

The gas samples that were analyzed were removed from the subsoil under a slight partial vacuum. In most cases no more than 10 cm. of mercury tension was used and often the average tension was less than that. Calculated from the equation $d = \dfrac{0.30}{h}$, the minimum diameter of pore[2] that could be emptied of water by a tension of 10 cm. of mercury would be about 20 μ. It seems conservative to assume, then, that only pores, cracks, and channels larger than about 20 μ in diameter delivered gas to the mercury pump in the early spring when soil moisture tension was less than 10 cm. of mercury. As the soil dried out, of course, some pores smaller than 20 μ in diameter allowed free diffusion of gas and yielded part of the gas sampled. But probably a very large proportion of the gas samples even from rather dry soil came from the larger soil pores.

If the samples indicate the seasonal changes in oxygen and carbon dioxide percentages of gas in the larger pores of these soils, the question arises: What was the situation in the part of the pore system not sampled? If the pores not sampled were filled with water or were sealed off by water from the larger pores,

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[2] The equation (6) is based on the assumption that the soil pores are circular in cross section.

there was no opportunity for free gas exchange with the outer atmosphere and any aerobic biological activity must have resulted in depletion of oxygen and building up of carbon dioxide. That is, essentially the same conditions would exist as occur in the larger pores normally in late winter and early spring as a result of accumulation of moisture in the soil.

If this is true, then the gas samples obtained give a picture of the oxygen and carbon dioxide levels in the aerated part of the pore system. It may be assumed that the rest of the pore system is under anaerobic conditions, or would be when biological activity occurred.

The aerated part of the pore system of a soil layer increases and decreases in volume inversely with its moisture content. As has been indicated in another paper (2), in some of these heavy subsoil layers the soil volume not occupied by water may increase from 1 per cent in early spring to 15 per cent in late summer. Since the compound aggregates shrink as they dry out, there are also important changes in pore and crack dimensions and in ramifications of the open-pore system as the growing season progresses. Unfortunately it has not been possible to estimate these changes in porosity in the course of this study. It should be recognized, however, that in the periods when oxygen percentages were lowest in gas samples from the heavy soils, the aerated pore space was at a minimum; and as oxygen percentages increased in the gas samples there was undoubtedly an increase in amount and ramification of aerated pore space.

A recent paper by Furr and Aldrich (8), who studied variations in subsoil oxygen and carbon dioxide percentages in an irrigated date orchard, gives further confirmation of the conclusion that changes in subsoil oxygen percentage are usually caused by changes in free pore space. Those investigators found the fluctuations of oxygen percentage in the upper subsoil to be associated with time and frequency of irrigation. They found at a depth of 8 feet, below the depth of irrigation, that the oxygen percentage was higher and the carbon dioxide percentage was lower than at 6-inch and 30-inch depths in soil that was wetted by irrigation. The study of Furr and Aldrich also confirms the conclusion that fluctuations of carbon dioxide percentages are normally less than those of oxygen percentages in soil gas samples from one location, and that the sum of O_2 and CO_2 percentages from a sample often deviates widely from 20.8 per cent of gas volume.

The value, for fruit growing, of heavy soils like the two studied may be different in regions of the Northeast with different normal rainfall. This study indicates that 6 inches of rain in a single month or 10 inches of rain in 2 months may cause a considerable drop in oxygen percentage or may delay the normal seasonal increase in oxygen percentage of gas samples from the subsoil layers. At Ithaca, New York, with average annual rainfall at 33 inches and average rainfall during the five growing-season months at 16.7 inches, long periods when monthly rainfall exceeds 4 inches are rare. In the western New York fruit area, average rainfall is very close to or less than that at Ithaca. But in the Hudson Valley fruit section with average annual rainfall 5 inches higher and a considerable part of the additional precipitation coming in the growing season, seasons of heavy rain-

fall are more frequent than in western New York. Thus it seems possible that a heavy soil like the "silty clay" soil studied might, over a period of years, be more satisfactory for fruit growing in the western New York area than in parts of the Hudson Valley area.

CONCLUSIONS

This study indicates that under the weather conditions in northeastern United States, oxygen percentage in gas from heavy subsoils is normally low in the early spring and increases as the season advances. Under these conditions the minimum oxygen level and the extent of the period of low oxygen pressure seem to be determined by accumulated precipitation, by soil texture and compaction, and by depth. Carbon dioxide percentage seems to fluctuate within a narrower range than oxygen, and seems, on the average, to reach maximum levels during the summer months when soil temperature is relatively high, even though oxygen percentage may be relatively high at the same time.

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RELIABILITY OF THE PRESSURE-MEMBRANE METHOD FOR EXTRACTION OF SOIL SOLUTION[1]

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A review of soil solution extraction methods and a description of a pressure-membrane method were presented in a previous paper (15). The preliminary physical results discussed at that time have been supplemented by a sudy of factors and conditions affecting the use of the pressure-membrane method for chemical investigations. The present paper describes subsequent modifications in the pressure-membrane apparatus, the present solution extraction technique, experimental data obtained with the method, and a comparison with the aqueous displacement method.

The method is based on the availability of hydrophilic cellulose membranes which, when wet and properly supported, withstand a large gas pressure difference without allowing the gas to stream through the pores. However, water or an aqueous solution in contact with the membrane on the high pressure side passes through to the low or atmospheric pressure side. If moist soil is placed in contact with the high pressure side of such a membrane, moisture will be removed from the soil and pass through the membrane. This process will continue until the surface force which retains water in soil (and which increases during moisture extraction) just balances the water extraction force established at the membrane by the pressure difference across the membrane. At equilibrium, the interface curvature of the water throughout the soil is equal to that at the membrane, and movement of water ceases.

The apparatus is similar to ultrafiltration cells used for filtration of colloids. Woodruff (20) has utilized conventional ultrafiltration apparatus for studying moisture retention by soils. The pressure-membrane apparatus provides more rapid delivery of water or solution from unsaturated soils by increasing the ultrafiltration area and thus reducing the height of soil through which the water must flow to the membrane. This apparatus has been used during the last 3 years both for soil solution studies and for investigating moisture retention by soils over wide ranges of moisture tension (16).

APPARATUS

It has been found that a 100-mesh brass screen provides better support for the membrane than the 80-mesh screen previously used. The removable $\frac{3}{16}$-inch

copper tubing connections between the apparatus and the gas source are made with flared brass couplings. Rubber gaskets $\frac{1}{8}$-inch thick are still being used, and occasionally they must be replaced. For the extraction of solutions through thin cellophane membranes, it has been found desirable to broaden the bearing surface of the bottom gasket, which rests on the membrane. For extraction chamber cylinders 12 inches outside diameter and $\frac{1}{4}$-inch wall, a gasket 12 inches outside diameter and 11 inches inside diameter effectively reduces tearing of the membrane.

The gas inlet connection on the upper plate consists of a $\frac{1}{8}$-inch pipe nipple attached to a $\frac{1}{8}$-inch L-fitting, into which a $\frac{3}{16}$-inch brass flared nipple is soldered. A differential pressure of 5 pounds per square inch above the rubber diaphragm
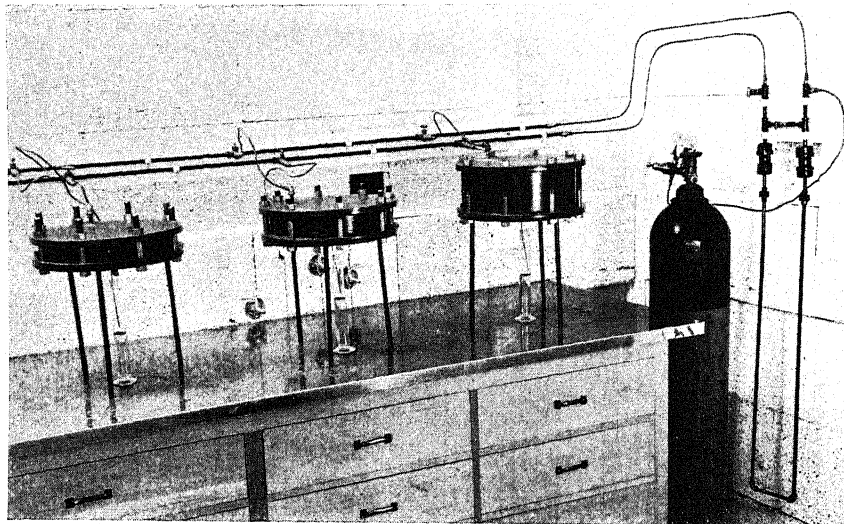
FIG. 1. ASSEMBLY OF THREE PRESSURE-MEMBRANE CELLS DURING EXTRACTION

is obtained from a mercury U-tube regulator with a by-pass needle valve inserted in the line between the gas source and the apparatus (16). These features can be observed in figure 1, which shows a group of three extraction cells attached to a nitrogen inlet manifold. The chambers shown are 1, 2, and 4 inches high. Eight complete cells have been assembled.

TECHNIQUE OF SOLUTION EXTRACTION

The circular brass plate with attached screen, the gasketed cylinder of desired height, and the soft rubber diaphragm are rinsed well with distilled water. When these are dry, the brass plate is laid on the tripod base plate. A sheet of cellophane, $12\frac{1}{4}$-inch square or larger, is placed on the screen. The cylinder is placed on the membrane and the cellophane trimmed to clear the bolt holes. The cylinder is then fastened to the tripod with the three thumb screws provided for this purpose.

The soil sample is placed on the cellophane in even layers, usually in several portions. Each layer is compacted slightly with a hand tamp several inches in diameter. The rubber diaphragm is then placed over the soil, the top steel plate is put into place, and the entire assembly is bolted tightly together with the eight $\frac{5}{8}$-inch steel bolts.

The two copper gas inlet tubes are connected to the manifolds, the upper tube on the high pressure side of the regulator. The open end of the delivery tube is inserted into a graduated glass mixing cylinder or other container. With the mercury regulator by-pass valve open, the pressure-reducing valve on the gas source is operated until the desired pressure is obtained in the extraction chamber. The by-pass valve is then closed and the needle valve vent on the mercury regulator opened until the mercury gurgles. The differential pressure of 5 pounds per square inch thus applied above the diaphragm prevents the drying soil from shrinking away from the membrane.

The time of appearance of the first portion of solution depends primarily on the moisture content of the soil, and for samples at field moisture usually varies from 10 minutes to 2 hours. The duration of the extraction depends on the quantity of solution required. A considerable flow of gas through the membrane indicates a leak, and the membrane may have to be replaced. There usually is a slight flow of gas from the cell, but this is due to passage of gas dissolved under pressure and its liberation under the membrane as atmospheric pressure is encountered.

EXPERIMENTAL METHODS AND RESULTS

Preparation of samples

Most of the soil samples used in this work were prepared from large field samples which had been passed through a $\frac{1}{8}$-inch sieve. Representative subsamples of these stock soils were thoroughly mixed by rolling and pulling on a Koroseal mixing cloth. They were moistened to predetermined moisture contents by sprinkling with distilled water after being layered into large tinned cans. Samples of other origin were treated in a similar fashion.

After preparation, and during storage in these air-tight containers, the samples were frequently removed and mixed by manual working of the soil particles followed by rolling on the mixing cloth. Most of the samples were stored in a constant temperature room at $21° \pm 0.5°C$. This reduces distillation of water from the soil caused by temperature gradients within the container. Most samples prepared to field moisture contents were stored in this manner for at least 2 weeks after addition of the water and were mixed every 2 to 4 days during this time. During this period of moisture equalization, changes may occur in the electrolyte composition from microbiological activities.

Soil samples of higher moisture content, for example at the liquid limit (upper plastic limit) and at the saturation percentage (17), appear to require a shorter period for equilibrium, presumably because of the more rapid mobility of the moisture at higher moisture contents.

Methods of analysis

To follow the possible changes in composition of a soil solution during the extraction process, the solution is collected in successive fractions for analysis. This procedure was started during the preliminary experimentation with the pressure-membrane method and has been followed ever since, even for routine extractions. The number of fractions usually ranges from three to five.

Only limited volumes of soil solution can be extracted from comparatively dry soils. When these volumes are further fractionated, accuracy of analysis may be seriously affected. To increase the precision of analysis in these cases, a set of semimicroanalytical methods has been developed for ions commonly determined in studies of salinity and fertility (14). The chemical results reported in this paper have been obtained by these methods and by macroanalytical methods used in the adjacent Rubidoux Laboratory (19).

Evaporation during prolonged extractions has been shown to be negligible, and consequently the solutions are collected in open cylinders, preferably graduated glass-stoppered mixing cylinders. The use of a device for the automatic separation and collection of fractions, such as that of Hibbard (7), has not been worked out satisfactorily for small volumes of solution.

Effect of temperature

It has been shown by Richards and Weaver (16) that an increase in the temperature decreases slightly the amount of water retained by a soil at a given pressure. Whether the composition of the soil solution during extraction might vary with temperature changes has not been investigated. To eliminate the possibility of such an effect, almost all the pressure-membrane extractions discussed in this paper were made in a room maintained at a constant temperature of $21° \pm 0.5°C$.

Pressure and composition of gas

The extent to which a soil on a membrane can be dried increases with the applied pressure. To obtain the necessary quantity of soil solution from a sample, the pressure must be great enough that a sufficient volume of extracted solution has been delivered when the soil moisture has approached equilibrium with it. The wilting range of soil moisture is a valuable consideration in studies on salinity, irrigation, and available moisture supply. The work of Richards and Weaver (16) and Furr and Reeve (6) has shown that for the great majority of soils the 15-atmosphere percentage lies in the wilting range between the first permanent wilting percentage and the ultimate wilting percentage. To obtain soil solutions at moisture contents near this range, a pressure of at least 15 atmospheres should be used. In most of the extractions on soils at field moisture, a pressure of 250 to 270 pounds per square inch (17 to 18 atmospheres) is used. For extractions involving higher moisture contents, such as the liquid limit or the saturation percentage, a pressure of 100 pounds per square inch is adequate.

Water-pumped nitrogen from commercial cylinder tanks was used as the source of pressure in the work reported here. During the recent shortage of commercial nitrogen, an air compressor was devised as a substitute. It appears that carbon

dioxide in the gas source or in the soil air may react with calcareous constituents of the soil to increase the concentrations of calcium, magnesium, and bicarbonate. A direct measurement of this effect on the composition of solutions obtained from calcareous soils by use of various gases has not been attempted, but a similar study has been made on an aqueous suspension of calcium carbonate. The results are presented in table 1.

Three portions of a 2 per cent suspension of c.p. calcium carbonate in distilled water were prepared and mixed on a reciprocating shaker. Each portion was transferred to a pressure-membrane cell having a $\frac{1}{2}$-inch cylinder and a sheet of water-washed No. 600 cellophane (see next section). The three suspensions were filtered under the indicated pressures of nitrogen, air, and carbon dioxide. The filtrates were analyzed in fractions for calcium, carbonate, and bicarbonate ions, and pH value.

The carbon dioxide in the compressed air appears to cause a very slight increase in the filtrate concentration. This effect, however, is of an entirely different

TABLE 1

Effect of gas composition on a suspension of calcium carbonate

GAS	PRESSURE	FILTRATION RATE	ION CONCENTRATIONS			pH RANGE
			Ca	CO₂	HCO₃	
	lb./sq.in.	*ml./min.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	
Nitrogen.............	148	1.83	0.25	0.00	0.42	7.6–8.1
Air..................	148	1.70	0.39	0.01	0.57	8.0–8.5
Carbon dioxide........	37	0.54	18	0.00	19	6.6–6.9

order of magnitude from that exerted by pure carbon dioxide. Some soil solutions have shown progressive parallel increases in calcium, magnesium, and bicarbonate concentrations. These result from an increase in soil carbon dioxide during the extraction, and will be discussed more fully in a later section. The effect appears important mainly with some calcareous soils of low soluble calcium content.

Membrane investigations

Several practical considerations are involved in the selection of a suitable cellulose membrane for solution extraction. Those discussed here include the following: general availability, permeability to liquids and to gases, progressive deterioration during the extraction, resistance to tears and cuts, and soluble inorganic and organic contaminants and their removal.

Membrane materials that have been tested and used are P. T. (plain transparent) cellophane[3] of gauge number 300, 450, and 600, and Visking cellulose sausage casing[4]. The latter material is especially satisfactory in the determination of 15-atmosphere percentage and in moisture sorption studies (16).

[3] E. I. du Pont de Nemours & Company, Wilmington, Delaware.
[4] The Visking Corporation, 6733 West 65th Street, Chicago, Illinois.

Cellophane membranes for ultrafiltration have been suggested and used by McBain and his co-workers (8, 9) for a number of years. They are almost universally available at low cost, and replace collodion membranes, which are difficult to prepare properly, especially in large sizes. Treatments for modifying the permeability of cellophane for various purposes have been described (8, 11).

Thickness measurements on recent supplies of Nos. 300, 450, and 600 cellophane and of Visking casing, respectively, are as follows: 0.00085, 0.00120, 0.00165, and 0.00395 inches. The cellophane values agree with those reported for previous samples (15). The decreased flow caused by increased membrane thickness is not very important in extractions from unsaturated soils, as the rate of flow in such cases is primarily determined by the mobility of the water through the soil. The permeability of the Visking casing to gas appears appreciably less than that of cellophane.

Because of its greater thickness, the Visking membrane usually withstands the conditions of extraction longer before serious gas leaks develop from membrane disintegration. The thinner membranes are also susceptible to immediate leaks when used with sandy soils, through cuts made by the sharp soil particles. This disadvantage has practically eliminated Nos. 300 and 450 cellophane as general utility membranes. They are also more apt to suffer tears during handling.

Appreciable quantities of ionic contaminants have been removed from membranes by pressure leaching and extraction with distilled water, as demonstrated by electrolytic conductivity measurements and analytical determinations. The most abundant ions appear to be sodium and chloride. The first fractions of many soil solutions show an initial excess of these two ions. This is not important for saline soils high in sodium, but may be for nonsaline soils. Consequently, the membranes now used are washed with water to remove the bulk of these impurities.

The present practice is to cover 15-inch squares of cellophane with distilled water in a large flat tray such as a photographic developing tray. The water is replaced daily for 10 to 15 days or until the conductivity has reached a minimum value. The membrane is then supported vertically to dry. McBain and Kistler (8) have mentioned that washed cellophane becomes brittle on drying, and this has been confirmed. Consequently, it is recommended that the washed membrane be air-dried immediately before placing it in the extraction cell. Following the water wash by a similar ethanol washing appears to decrease the brittleness, but the extra procedure usually is not necessary to obtain a suitably strong membrane.

In addition to the ionic contaminants, the first fractions of a leachate through an unwashed Visking membrane contain organic material leached from it. On evaporation a dark gummy deposit is formed, which requires continuous drying at 105°C. for several days to reach constant weight. This contamination seriously affects the determination of osmotic pressures and other colligative quantities.

Consideration of these various factors has led to the acceptance of water-washed No. 600 P.T. cellophane as the choice for an all-around membrane for solution extraction.

Rate of extractions

It is theoretically possible to extract soil solutions at any gas pressure that the apparatus can withstand. To obtain solution from a soil containing moisture at a tension of 15 atmospheres, it is necessary to apply a pressure greater than this value. Extractions at this moisture content are repeatedly made in moisture sorption studies, and have been accomplished for soil solution work. As would be expected, however, a decrease in the initial moisture content diminishes the volume of water available for extraction and consequently decreases the rate of extraction.
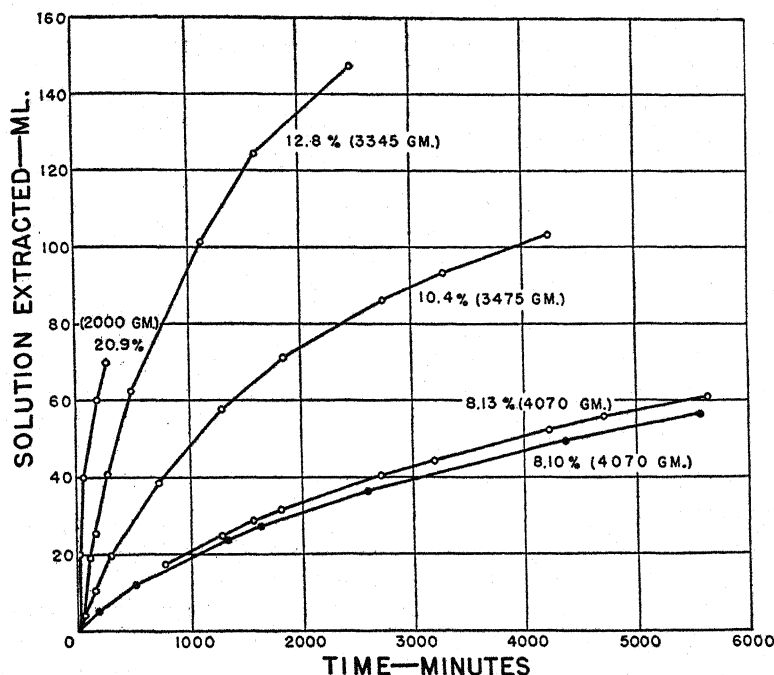
Fig. 2. Effect of Moisture Content on Rate of Solution Extraction from Hesperia Sandy Loam

The dependence of the extraction rate of Hesperia sandy loam on the initial moisture content is illustrated by the cumulative extraction curves in figure 2. This soil has a 15-atmosphere percentage of 4.7 and a one-third-atmosphere percentage of 10.6. The latter tension value has been shown by Richards and Weaver (16) to approximate the moisture-equivalent value for most soils. The figures adjacent to the curves represent the initial moisture contents of the various samples, and the figures in parentheses, the initial weights. All moisture percentages are based on soil oven-dried at 105°C. The gas pressure was 270 pounds per square inch.

The two 8 per cent extractions were made 17 days apart, and their similar nature illustrates the reproducibility of extraction rates. These results are typi-

cal of those obtained for other soils. In all extractions in which the rate is not primarily determined by the permeability of the membrane, the rate decreases continuously during the entire extraction period. The minimum moisture content at which a soil is extracted often depends on the volume of solution required or on the time available for obtaining it.

Uniformity of composition

Previously reported investigations of the displacement method (3, 13) and the direct-pressure method (4) have shown that solutions can be obtained which

TABLE 2

Reaction and moisture characteristics of soil samples

ACCESSION NUMBER	SOIL TYPE	LOCATION	DEPTH	pH	HCl TEST	15 ATM. PERCENTAGE	½ ATM. PERCENTAGE
			in.			*Pw*	*Pw*
56	Imperial clay	Meloland, California	6–18	7.8	vigorous	15.1	29.7
57	Imperial clay	Imperial, California	6–18	7.2	vigorous	20.4	36.2
58	Indio very fine sandy loam	Coachella, California	6–18	8.9	vigorous	6.1	21.6
61	Billings clay	Bridgeland, Utah	6–18	8.0	vigorous	11.7	21.4
62	Oasis clay loam	Delta, Utah	2–6	7.7	vigorous	8.8	25.1
63	Oasis clay subsoil	Delta, Utah	8–12	8.0	vigorous	16.8	33.7
66	Millville loam	Logan, Utah	2–6	7.9	mild	7.5	22.6
68	Silty clay loam	Vale, Oregon	2–6	10.0	mild	14.6	35.6
77	Superstition sand, silted phase	Yuma, Arizona	2–6	8.1	vigorous	6.6	12.5
79	Cajon silt loam	Glendale, Arizona	2–6	7.7	mild	12.0	28.2
80	Gila silt loam	Buckeye, Arizona	2–6	8.8	mild	14.2	27.7
84	Gila adobe clay	Las Cruces, N. Mexico	2–8	8.0	mild	22.8	41.2
85	Reagan clay loam	Roswell, N. Mexico	2–8	7.8	vigorous	11.4	23.2
86	Fort Collins loam, poorly drained phase	Laramie, Wyoming	1–6	8.0	mild	11.8	22.4
91	Fallbrook loam	Riverside, California	6–12	7.6	trace	6.1	16.0
183	Hesperia sandy loam	Shafter, California	0–8	7.9	trace	4.7	10.6

remain practically constant in composition during the extraction. Over the moisture ranges investigated, the pressure-membrane method also furnishes substantially uniform solutions during extractions of practical duration.

Characteristics of soil samples 57, 66, 84, 85, and 183, used as examples, are presented in table 2, and include pH values at the saturation percentage, without regard to carbon dioxide content, and effervescence on addition of 6 N HCl. Tables 3 and 4 contain compositional data obtained on these soils. In table 5 is listed various information concerning the seven extractions involved, apart from the composition of the solutions. None of these extractions was continued to equilibrium moisture conditions, as shown by the fact that the final moisture

content of the top section of the soil was appreciably higher than that of the bottom section nearest the membrane. Sample 85 C was extracted without a rubber diaphragm above the soil. In an examination of the material of tables 3 and 4 it should be considered that minor variations may result from analytical errors. Also, the instability of carbonate and nitrogen systems often induces serious disagreement, and nitrate results are omitted from some listings.

In addition to remaining uniform throughout an extraction, a satisfactory solution should be representative of the moisture content at which it is extracted. That is, in general, over narrow moisture ranges, the concentration of soluble

TABLE 3

Composition of soil solutions extracted from Reagan clay loam at three moisture contents

EXTRACT FRACTION	VOL-UME	RATE OF FLOW	pH	ELECTRICAL CONDUC-TIVITY*	IONIC CONCENTRATIONS						
					Ca	Mg	Na	K	CO_3-HCO_3	SO_4	Cl
	ml.	*ml./min.*		$K \times 10^5$	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*
85 A—14.2 per cent											
1	12	0.0228	8.36	...	43.1	50.0	29.1	11.2	4.1	43.2	29.4
2	14	0.0148	8.03	...	47.6	60.0	29.8	11.2	9.2	46.6	29.9
3	12	0.0110	8.13	...	47.9	65.3	30.1	11.4	13.5	47.5	29.7
4	14	0.0084	8.15	...	47.6	65.7	30.4	11.7	10.4	49.3	30.3
85 B—20.0 per cent											
1	22	0.122	8.10	940	42.2	47.3	24.1	...	2.9	57.9	19.1
2	32	0.091	8.32	952	44.2	49.3	22.2	9.6	4.9	57.6	19.5
3	35	0.063	8.25	956	41.6	50.4	22.6	9.4	5.9	58.1	20.2
4	24	0.044	8.38	956	44.0	53.0	22.1	9.7	7.1	57.8	20.2
5	29	0.030	8.37	961	44.1	52.3	22.2	9.6	7.6	56.2	20.2
6	27	0.017	8.26	940	43.5	48.0	21.7	9.6	7.2	56.3	20.1
85 C—32.5 per cent											
1	25	1.57	7.78	...	26.7	30.5	15.1	6.1	7.2	59.0	12.4
2	25	1.47	7.97	...	29.0	34.2	15.2	6.3	8.4	63.0	11.9
3	50	0.93	7.86	590	28.3	34.5	14.8	6.3	8.7	63.5	11.9
4	53	0.28	8.22	573	28.4	33.9	14.6	6.3	8.6	62.3	11.3

* At 25°C.

salts should be inversely proportional to the moisture content. Table 3 shows that for Reagan clay loam an extracted solution is characteristic of only one moisture percentage and differs from solutions obtained at other moisture contents. By reference to table 2, A and B are seen to represent moisture values between wilting range and moisture equivalent, whereas C represents the "liquid limit" as determined by 10 impacts of the Bodman and Tamachi (2) procedure.

Several types of variation encountered also in table 4 can be observed here. Calcium, magnesium, and sulfate concentrations in the initial fractions are usually lower than in the subsequent fractions. Sodium, chloride, and potassium

sometimes are higher in the initial fraction, presumably from residual contamination in the washed cellophane. By discarding the values obtained from analysis of the first fraction, substantially uniform acceptable values for these six ions are almost always obtained. This suggestion is in accord with the common practice

TABLE 4

Composition of soil solutions extracted from four soils

EX-TRACT FRAC-TION	VOL-UME	RATE OF FLOW	pH	ELECTRICAL CONDUC-TIVITY*	IONIC CONCENTRATIONS							
					Ca	Mg	Na	K	CO_3-HCO_3	SO_4	Cl	NO_3
	ml.	*ml./min.*		$K \times 10^5$	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*
Imperial clay (No. 57)—27.2 per cent												
1	15	0.099	....	....	750	315	....	2.9	3.1	18.6	1490	85
2	15	0.087	....	....	785	325	570	3.0	3.4	19.4	1555	88
3	22	0.061	....	....	790	318	559	2.9	4.4	19.0	1555	89
4	27	0.050	....	....	785	318	560	2.9	4.0	19.3	1550	89
5	..	.....	....	....	787	315	566	2.9	3.4	20.0	1555	89
Gila adobe clay (No. 84)—28.9 per cent												
1	18	0.044	6.55	543	23.9	6.1	23.6	1.0	1.2	23.3	25.7	..
2	23	0.025	7.52	478	23.4	5.9	22.7	1.0	2.1	25.7	16.7	..
3	24	0.017	8.12	466	23.4	6.0	22.4	1.0	3.7	25.6	14.6	..
4	19	0.013	8.07	472	23.2	6.1	23.0	1.0	4.4	26.2	14.6	..
5	26	0.009	7.87	475	24.4	6.1	22.8	0.9	4.5	26.7	14.9	..
Millville loam (No. 66)—13.6 per cent												
1	17	0.064	7.69	180	9.8	5.2	4.3	0.37	1.7	1.9	5.1	..
2	40	0.043	8.14	172	12.7	6.2	1.3	0.22	4.1	1.3	3.1	..
3	24	0.026	8.28	178	14.0	7.0	1.0	0.23	5.5	1.4	3.0	..
4	26	0.013	8.35	188	14.2	7.2	1.7	0.26	6.7	1.3	3.1	..
Hesperia sandy loam (No. 183)—10.4 per cent												
1	11	0.073	7.7	279	13.7	1.9	5.6	...	1.1	3.2	2.9	18.2
2	9	0.059	7.6	278	15.7	2.7	3.3	...	1.2	3.4	3.1	17.8
3	19	0.046	8.3	284	17.2	2.9	3.4	1.6	2.2	2.9	3.3	16.6
4	20	0.033	8.4	289	18.3	4.4	3.3	1.7	3.8	2.8	3.4	18.6
5	13	0.024	8.4	297	19.5	4.7	3.6	1.7	4.9	2.8	3.5	17.4
6	15	0.017	8.4	303	20.2	5.2	3.1	1.7	5.6	2.6	3.3	17.4

* At 25°C.

of discarding the initial portion of a filtrate. In table 3 the sulfate concentration is seen to increase with the moisture content, instead of decreasing. This soil is gypsiferous and the soil solution is saturated with gypsum even in a 1:5 extract. The increase in sulfate is evidently related to the concomitant decrease in calcium concentration [see Vanoni and Conrad (18)]. Several other gypsiferous soils have been observed to act similarly. The carbonate values are also

unrelated to the moisture content, because of the effects of such factors as carbon dioxide pressure, presence of calcium and magnesium carbonates, and composition of the soluble salts.

Table 4 includes results for four soils which were selected to show possible effects of texture, moisture content, and salinity on the versatility of the method. In the order listed, they include a fine-textured very saline soil, a fine-textured nonsaline soil, a medium-textured nonsaline soil, and a coarse-textured nonsaline soil. For the four soils, the ratios of initial moisture percentage to 15-atmosphere percentage were 1.33, 1.27, 1.81, and 2.21, respectively. For the Reagan soil of table 3, the ratios were 1.24, 1.78, and 2.85, respectively.

The same general uniformity of composition characteristic of table 3 is apparent here. Again, the ions that were abnormally high or low in the initial fractions show the same tendencies in these soils. Some of these solutions provide examples of the carbonate effect mentioned previously, i.e., the parallel

TABLE 5

Conditions of operation involved in the extractions of tables 3 and 4

ACCESSION NUMBER	INITIAL WEIGHT WET SOIL	FINAL MOISTURE CONTENT		DURATION OF EXTRACTION	PRESSURE	CYLINDER HEIGHT	MEMBRANE
		Top	Bottom				
	gm.	Pw	Pw	hours	lb./sq. in.	in.	
57	5000	25.1	24.9	21	250	4	Unwashed 600 cellophane
66	3000	9.4	8.5	68	265	1½	Washed 600 cellophane
84	4000	25.8	21.8	118	255	2	Washed 600 cellophane
85A	4240	12.8	11.4	70	270	2	Washed 600 cellophane
85B	3500	13.8	12.9	70	270	1½	Washed 600 cellophane
85C	2040	22.8	19.8	5	270	1	Washed 600 cellophane
183	3475	7.0	5.7	71	270	1½	Washed 600 cellophane

increase in calcium, magnesium, and carbonates during the extraction. This effect is especially noticeable in Hesperia sandy loam, in which the bicarbonate increase is accompanied by regular increases in calcium and magnesium throughout the entire extraction period. It has been noticed also in several displacement extractions under pressure. The effect is explained by an increase in carbon dioxide in the chamber resulting from microbiological respiration. When the moist soil sample is mixed just prior to the extraction, much of the carbon dioxide accumulated during storage is lost to the atmosphere. During the extraction, the microbiological processes again increase the carbon dioxide pressure, with a corresponding progressive increase in solubility of calcium and magnesium carbonates. In such cases the choice of the correct concentrations of the ions involved must be somewhat arbitrary, and preferably based on the projected use of the data.

In addition to the ions discussed, phosphate and silica have been studied to some extent. With due allowance for analytical errors, silica appears to be extracted at a uniform concentration. The method is not satisfactory for phosphate. Extracted solutions usually indicate absence of phosphate, although

measurable amounts of soluble phosphate are shown by other means. Synthetic solutions of primary, secondary, and tertiary potassium phosphates have been filtered through cellophane under pressure; each filtrate showed a drastic initial reduction in phosphate concentration to about 1 per cent of the correct value, and a subsequent slow regular increase, whereas the concentration of the unfiltered portion increased considerably above that of the original solution. This behavior accords with the difficulty of filtering phosphate through other filtration media, and renders the pressure-membrane method useless for this constituent. If the phosphate concentration is appreciable, the accompanying cations may also be affected.

McBain and co-workers have made detailed studies of the filtration of colloids, electrolytes, and nonelectrolytes through cellophane. McBain and McClatchie (9) showed that uniform composition of an ultrafiltrate throughout the filtration is not absolute proof that it is representative of the intermicellar liquid. Their work on ferric hydroxide sols and the work of McBain, Kawakami, and Lucas (10) on soaps demonstrate an inverse relationship between concentration and rate of filtration. McBain and Stuewer (11) showed that the retention of solute by a membrane varies directly as the rate of stirring and inversely as the concentration. These results were explained on the basis of Donnan equilibria existing between a colloid and its intermicellar liquid, and between the membrane and the solute.

These phenomena might affect the application of the pressure-membrane method. Consideration of the differences between the method and ultrafiltration leads to several hopeful conclusions. The absence of stirring precludes difficulties from this source. The rate of flow is very low compared to ultrafiltration rates. In a pressure-membrane extraction of an unsaturated soil, the solution reaching the membrane passes through it and does not accumulate, which obviates the ion and colloid diffusion processes that influence ultrafiltration. In concentrated solutions, these effects should be further minimized.

To obtain a measure of the maximum possible effects of these processes on the filtration of the particular ions studied in this work, several synthetic combinations of calcium, magnesium, sodium, potassium, bicarbonate, sulfate, chloride, and nitrate, each at a concentration of 5 m.e. per liter, have been filtered through cellophane. Only calcium and sulfate appeared to be significantly affected; the initial concentrations of these ions were low, and increased during the filtration. Under these conditions, any effect is exaggerated, and no serious trouble is anticipated from the actual method. It is possible that the initial reduction in many ions during most extractions is a transient Donnan effect, and this difficulty is usually avoided by discarding the initial fraction.

Comparison with the aqueous displacement method

The questions raised in the preceding section concerning the lack of significance of a uniform composition in ultrafiltration make it desirable to compare the method with another independent method of extraction. For this purpose, the aqueous displacement procedure of Burd and Martin (3) was employed. The

TABLE 6

Comparison of pressure-membrane and displacement methods

METHOD	VOL-UME	AVER-AGE RATE OF FLOW	pH	IONIC CONCENTRATIONS							
				Ca	Mg	Na	K	CO₃-HCO₃	SO₄	Cl	NO₃
	ml.	*ml./min.*		*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*

Imperial clay (No. 56)—29.9 per cent

METHOD	VOL-UME	RATE	pH	Ca	Mg	Na	K	CO_3-HCO_3	SO₄	Cl	NO₃
Pressure-membrane..	425	0.077	8.2	30.3	15.0	29.9	...	4.5	54.9	14.2	6.5
Displacement.........	21	0.02	7.6	31.2	14.8	29.6	...	2.5	52.6	15.2	....

Imperial clay (No. 57)—34.8 per cent

Pressure-membrane...	399	0.255	7.7	584	247	439	2.7	1.8	29.5	1173	66
Displacement.........	35	0.01	7.2	558	246	435	2.9	1.8	32.7	1147	60

Indio very fine sandy loam (No. 58)—16.5 per cent

Pressure-membrane...	91	0.053	9.3	3.2	0.5	70.8	1.5	9.9	41.3	19.1	6.9
Displacement.........	80	0.133	8.6	3.9	0.5	69.8	1.5	5.7	44.3	19.2	7.4

Indio very fine sandy loam (No. 58)—19.7 per cent

Pressure-membrane...	86	0.118	9.6	2.2	0.5	58.0	1.2	9.3	33.8	11.3	5.6
Displacement.........	59	0.02	8.7	2.2	0.5	56.4	1.4	6.0	37.6	11.9	5.9

Billings clay (No. 61)—17.7 per cent

Pressure-membrane...	51	0.007	8.3	11.1	3.5	5.4	0.4	2.3	8.5	3.0	0.2
Displacement.........	100	0.001	8.4	8.0	3.7	5.3	0.4	3.0	9.7	3.3	0.2

Oasis clay loam (No. 62)—21.9 per cent

Pressure-membrane...	393	0.071	...	56.5	147	2002	65.9	6.2	233	1999	14.1
Displacement.........	72	0.058	8.1	63.1	154	2002	61.6	6.5	239	2024	....

Oasis clay subsoil (No. 63)—29.4 per cent

Pressure-membrane...	357	0.228	8.4	39.0	96.6	841	21.5	5.6	145	860	1.6
Displacement.........	31	0.007	8.2	41.4	93.9	847	22.4	5.5	153	870	....

Silty clay loam (No. 68)—29.3 per cent

Pressure-membrane...	98	0.057	9.8	0.3	0.1	1110	36	390	500	277	7.6
Displacement.........	194	0.072	9.7	0.4	tr	1130	36	383	505	285	7.8

Superstition sand (No. 77)—14.5 per cent

Pressure-membrane...	85	0.020	8.4	11.1	4.7	11.2	0.4	8	9.7	4.9	....
Displacement.........	63	0.004	8.7	14.4	4.7	11.1	0.7	8	10.4	4.9	....

TABLE 6—*Continued*

METHOD	VOLUME	AVERAGE RATE OF FLOW	pH	IONIC CONCENTRATIONS							
				Ca	Mg	Na	K	CO_3-HCO_3	SO_4	Cl	NO_3
	ml.	ml./min.		m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.
Cajon silt loam (No. 79)—24.8 per cent											
Pressure-membrane...	90	0.052	8.2	28	15.4	20	2.2	8	17.5	10.4	30
Displacement.........	174	0.011	8.5	28	14.8	19	2.3	8	18.8	11.2	29
Gila silt loam (No. 80)—26.3 per cent											
Pressure-membrane...	77	0.048	9.1	1.7	1.7	471	1.7	20	421	43	....
Displacement.........	222	0.005	8.6	1.8	1.8	488	1.7	15	442	44	....
Reagan clay loam (No. 85)—20.4 per cent											
Pressure-membrane...	85	0.029	8.3	44	51	23.5	9	7	54	19.8	44
Displacement.........	179	0.006	8.6	45	50	23.0	9	11	59	19.8	....
Fort Collins loam (No. 86)—20.1 per cent											
Pressure-membrane...	56	0.013	8.5	24	215	157	3.1	20	335	24	0.2
Displacement.........	48	0.002	8.4	23	238	165	3.3	22	358	25	0.3
Fallbrook loam (No. 91)—13.7 per cent											
Pressure-membrane...	80	0.055	8.5	12	4.0	3.5	0.2	5	3.8	1.9	....
Displacement.........	112	0.072	8.7	14	4.1	3.2	0.3	7	4.4	1.9	....

apparatus used was that of the adjacent Rubidoux Laboratory, similar to the original equipment and described briefly by Eaton and Sokoloff (5). Fourteen soil samples have been extracted by the two methods, including extractions at two moisture contents on Indio very fine sandy loam.

The results are presented in table 6. Characteristics of the soil samples are given in table 2. Many of the displacements had to be repeated because of early contamination with displacing liquid (0.1 N potassium thiocyanate), by channeling or similar process. Some displacements were exceedingly slow, as shown by the rates of flow, and were continued for extended periods of time to ensure adequate samples. Displacement pressures varied from 0 to 100 pounds per square inch in accordance with the permeability of the soil. Because solutions cannot be displaced from many soils at relatively low moisture contents, these samples were planned to be near the moisture equivalent. As in the pressure-membrane extractions, the displaced solutions were analyzed in fractions.

In general, the comparisons are seen to be satisfactory, and for most practical purposes identical. Discrepancies, some minor and some more serious, have occurred. The number of significant figures reported for a value provides a

rough index of the probable accuracy. Some nitrate values are omitted because lack of agreement on different fractions precluded accurate evaluation. Displacement nitrate values often decrease during the extraction, sometimes to zero concentration, and this is usually attributed to denitrification processes under anaerobic conditions. Bicarbonate-carbonate values show variations, a phenomenon which is to be expected from the carbon dioxide pressure effect. Soils 61, 77, and 91 probably show the most serious disagreement, and these represent the least saline samples. The displaced solution from soil 85 showed serious decreases in all ions during extraction, but the values for the initial fraction, reported here, compare very favorably with those of the pressure-membrane method.

Except for the variations indicated, it is considered that the two methods provide substantially the same soil solution. In the pressure-membrane method, the soil moisture is continuously depleted during the extraction, whereas in the displacement procedure the moisture is replaced by the displacing liquid; despite this fundamental difference in principle, the same solution appears to be delivered by both techniques. The severe ramming and tamping required by the displacement method puddles the soil sample, but in the pressure-membrane method the structure usually is not seriously disturbed by its insertion into the apparatus; notwithstanding this difference, substantially the same solution is obtained.

These comparative results are considered reliable evidence that the ultrafiltration effect is of slight consequence in the pressure-membrane method. In addition, when the same solution is extracted from a soil by two independent methods based on different principles, assurance is provided that the results are characteristic of the soil and not of the method.

DISCUSSION

The existence in macroscopic soil masses of solutions homogeneous in composition throughout the entire thickness of soil moisture film is now generally regarded as untenable. The surface activity of soil colloids, involving the exchange of cations, and possibly of anions, presumably establishes ionic concentration gradients over finite distances from the surfaces of the finer soil particles. In accordance with this theory, an extracted solution homogeneous in composition throughout the extraction might not be anticipated.

In general, however, soil solutions extracted by the pressure-membrane procedure remain constant in composition during the extraction. This accords with results reported for the displacement and direct-pressure methods. Though the moisture film removed by these several methods is of homogeneous composition, the unremoved portion may be affected by the colloidal forces. It is possible that this question may be answered, at least in part, by a further application of the pressure-membrane technique. This would involve the fractional stepwise extraction of solutions at increasing moisture tensions, as in sorption curve studies, continuing the extraction to the lowest practical moisture level.

Variations in composition should be more important in the case of dilute solu-

tions.   With regard to this effect in colloidal systems, McBain and McClatchie (9) say: "Variations in the concentration of the ultrafiltrate would be much less for sols with a higher ratio of simple electrolyte to colloidal electrolyte, for a Donnan equilibrium would then produce only a slight inequality in the distribution of the simple electrolyte."

The pressure-membrane method has operated satisfactorily on soils involving wide ranges of moisture content, salinity, texture, and structure.   Extractions have been made at moisture levels from the wilting range to 1:5 extracts, but the method is not recommended for moisture contents above saturation, as other available means are more convenient.   Dilute solutions usually are more susceptible to effects of membrane and pressure than concentrated solutions, and divalent ions are influenced more than are monovalent ions.   Phosphate is seriously affected.   Fine-textured soils are effectively extracted by this procedure.   Coarse-textured soils, such as sandy loams, cannot be satisfactorily extracted at the relatively low moisture contents (relatively high tensions) adequate for fine-textured soils, for such reasons as insufficiency of available solution, reduced number of soil-membrane contact points, and damage to the membrane.

The method has been used successfully in several reported investigations (1, 12) and in unpublished work.

SUMMARY

Investigations of the pressure-membrane method for the extraction of soil solution are reported.   The technique of operation, recent modifications in the apparatus, and factors affecting the method, such as pressure and composition of the gas, choice of a suitable membrane, and rate of extraction, are discussed.

Solutions obtained from a variety of soils remain uniform during the extraction period.   Minor deviations in some ions may result from membrane filtration effects and the presence of insoluble carbonates, but these can be minimized or eliminated by analysis of the extract in fractions.   At each moisture content a solution is obtained which is representative of that condition.   Comparisons with the aqueous displacement method on thirteen soils indicate that the two methods supply substantially identical solutions.   The method is adapted to wide ranges of moisture, salinity, and texture.

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THE RELATIVE AVAILABILITY TO PLANTS OF EXCHANGEABLE CALCIUM FROM SOIL SEPARATES OF SAND, SILT, AND CLAY[1]

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It has been recognized for some time that adsorbed and chemically exchangeable cations held by the soil colloidal complexes are available to plants as nutrients. Nostitz (10), using permutite saturated with various cations, indicated that plants were able to use such adsorbed ions. That calcium in the exchange complex of soil colloidal materials is available to plants has been shown by Joffe and McLean (7), Gedroiz (2), Joffe (8), Albrecht and McCalla (1), and others. These studies have dealt primarily with the clay fractions of the soil. Although the larger sand and silt fractions constitute more than 80 per cent of most surface soils, they have generally been considered largely inert, a view held by Russell (11), with respect to supplying nutrients for plants. Recently, however, Graham (3, 4, 5) has shown that certain soil particles of silt- and sand-size, after being weathered artificially by treatment with hydrogen-clays, can furnish available cations for plants.

This paper deals with the relative availability to plants of exchangeable calcium from sand, silt, and clay soil fractions after saturation with calcium ions but without subsequent "weathering" treatment.

EXPERIMENTAL PROCEDURE

The soil separates of sand, silt, and clay were obtained from the B_2 and C horizons of a Montalto silt loam profile found on the basalt of Watchung Mountain in central New Jersey. The approximate limits in size of the particles in each fraction were: sand, > 50 μ; silt, 50 to 2 μ; and clay, < 2 μ.

The separates were prepared for experimental use by continuous leaching first with calcium acetate buffered to pH 7.0 and then finally with distilled water to remove excess calcium ions not held in exchangeable form. The amount of calcium remaining in exchangeable form varied from 0.125 m.e. per gram in the silt fraction to 0.379 m.e. per gram in the clay fraction. To test the availability to plants of their adsorbed calcium ions, sufficient amounts of each of the separates were weighed out in triplicate so that each sample contained 100 mgm. of calcium in exchangeable form. This amount was chosen because it was estimated, on the basis of the growth made by plants in a recent somewhat similar experimental set-up (9), that it would be adequate to provide the necessary calcium for the growth of the plants for the duration of the experiment. The

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size and the exchangeable calcium content of soil-separate samples prepared
for experimental use are given in table 1.

Each weighed sample was then thoroughly mixed with 1900 gm. of white
quartz sand that had been washed with acid, alkali, and distilled water, and the
mixtures were placed in porcelain pots having a high silica glaze. The drainage
holes were tightly sealed with rubber stoppers.

A sufficient amount of nutrient solution containing all essential elements other
than calcium was then added to each culture to bring the whole mixture up to a
desirable moisture content. As controls, similar cultures containing only white
quartz sand were prepared in triplicate and to each was added a nutrient solution
similar to that used with the other cultures with the exception that the 100 mgm.

TABLE 1

*Exchangeable calcium present and size of each sample of soil-separate fractions required to
yield 100 mgm. of calcium*

SOIL SEPARATE	EXCHANGEABLE CALCIUM	CALCIUM IONS	SAMPLE REQUIRED TO YIELD 100 MGM. CALCIUM IONS
	m.e./gm.	*mgm./gm.*	*gm.*
Sand............................	0.272	5.44	18.38
Silt............................	0.125	2.50	40.00
Clay............................	0.379	7.58	13.06

TABLE 2

*Composition of nutrient solutions used to determine the availability to tomato plants of soluble
calcium and of exchangeable calcium from soil separates of sand, silt, and clay*

NUTRIENT SOLUTION	CONCENTRATION OF SALTS				CONCENTRATION OF TRACE ELEMENTS			
	KH₂PO₄	Ca(NO₃)₂	NaNO₃	MgSO₄	Fe	Mn	B	Zn
	M	M	M	M	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
With soluble calcium.......	.00263	.00526	......	.00263	0.5	0.25	0.5	0.5
Lacking calcium, for soil-separate cultures........	.00263	......	.01052	.00263	0.5	0.25	0.5	0.5

of calcium ions were added to these cultures in soluble form as calcium nitrate.
The composition of each nutrient solution is given in table 2.

Three small Marglobe tomato seedlings selected for uniformity were planted
in each pot on October 27, 1941. The water content of each culture was main-
tained at the desired point by daily additions of distilled water.

After 2 weeks, slight symptoms of iron deficiency appeared on plants growing
in the control cultures containing soluble calcium. At this time a supply of the
trace elements iron, boron, manganese, and zinc equal in amount to that origi-
nally present in the solution was added to each culture. As a result, the green
color of the leaves was partly restored, though it did not quite equal in intensity
that of plants growing in the other soil-separate cultures. No additions of other
essential nutrients were made.

At the end of 6 weeks the plants in all the cultures showed by slight but characteristic deficiency symptoms that the available supply of nitrogen was gradually becoming a limiting factor to further growth.   No symptom of calcium deficiency was apparent in any of the cultures.   The plants were then harvested and their green and dry weights and calcium contents determined.

RESULTS AND DISCUSSION

One of the most interesting results of this experiment is the fact that the plants in the sand-separate cultures grew just about as well as those in the silt- and clay-separate cultures.   As shown in table 3, the average green weight of the tops of the plants in the sand-separate cultures was 20.4 gm., in the silt-separate cultures 22.2 gm., and in the clay-separate cultures 19.2 gm.   The ash-free dry weights

TABLE 3

Average green and dry weights and calcium and ash contents of tomato plants grown in sand cultures with soluble calcium and with exchangeable calcium from soil separates of sand, silt, and clay

FORM OF CALCIUM SUPPLIED	FINAL pH OF SUB-STRATE SOLUTION	TOPS					ROOTS			WHOLE PLANTS
		Green weight per plant	Dry weight per plant	Calcium content of dry matter	Calcium re-moved per plant	Ash content	Dry weight per plant, ash-free	Calcium content of dry matter	Calcium re-moved per plant	Total calcium removed per plant
		gm.	*gm.*	*per cent*	*mgm.*	*per cent*	*gm.*	*per cent*	*mgm.*	*mgm.*
Soluble...........	6.8	10.1	0.85	3.85	32.7	21.7	0.19	2.15	3.2	35.8
Exchangeable, sand-separate...	7.1	20.4	1.68	1.51	25.3	17.8	0.50	0.54	2.7	27.9
Exchangeable, silt-separate....	7.2	22.2	1.91	1.44	27.4	17.7	0.50	0.51	2.5	29.9
Exchangeable, clay-separate...	7.3	19.2	1.66	1.66	27.7	19.9	0.41	0.67	2.7	30.1

of the roots of these respective cultures also differed but little from one another. Likewise, when considered on the basis of the ash content and calcium content of the tissues, there appeared to be no great differences between the mineral metabolism of these cultures.   The plants in the sand-separate cultures absorbed 27.9 mgm., in the silt-separate cultures 29.9 mgm., and in the clay-separate cultures 30.1 mgm. of calcium per plant.

The plants in the cultures supplied with calcium in soluble form as calcium nitrate behaved quite differently from those in the various treatments containing the soil-separate fractions.   Their average green and dry weights per plant were approximately only half as great, whereas their ash and calcium contents, expressed in percentage of the dry matter, were much greater than the corresponding values in the soil-separate cultures.   This indicates a so-called "luxury consumption" of calcium and at the same time emphasizes the effectiveness of large as well as small soil particles bearing exchangeable calcium ions in the process of absorption by roots and in green and dry weight production of plant

tissue. The low green and dry weight production of the plants supplied with calcium in the form of the nitrate was undoubtedly associated with the chlorosis which, as previously noted, appeared during the early growth phases of the experiment. This chlorosis was of the iron-deficiency type which is associated with an increase in pH values of the nutrient solution and is likely to occur in sand or solution cultures when nitrogen is supplied solely in the nitrate form and particularly when, as in this experiment, iron is not added to the cultures daily. The maintenance of chlorosis-free growth in the soil-separate cultures is probably to be accounted for as part of the "regulatory action" which soil-colloidal substances possess and which is so important in the adjustment of plants to varying conditions in the soil.

The fact that quantities of calcium of the same order of magnitude were removed by plants from the three series of soil-separate cultures brings up an interesting point regarding the mechanism by which an exchangeable ion is absorbed by plants. The means by which an ion, held in exchangeable form on a soil particle, arrives at the root surface has long been a subject of speculation. The older point of view is that some ion in the soil solution, usually hydrogen, replaces the exchangeable cation, like calcium, which then migrates toward the root surface. The hydrogen ion may come from the root surface itself. Jenny and Overstreet (6) have proposed a somewhat different mechanism for the absorption of exchangeable ions. According to this theory, termed "contact exchange," the exchangeable ion on the soil particle need not leave the sphere of influence of the particle before it reaches the root surface and is absorbed. If the root surface comes in contact with the particle, the exchange occurs between the exchangeable ions of the particle and the root surface. The results herein reported would seem to throw some light on the question whether the absorption of exchangeable calcium ions, in this experiment at least, took place largely by a contact exchange mechanism. If this were so it would be expected that the greatest absorption of calcium ions would have occurred in the cultures with the clay fractions, which, on the basis of particle size, possess the greatest area of exposed surface. There was little difference, however, between the total exchangeable calcium removed by the plants in the clay-, silt-, and sand-separate cultures.

Of course, as table 1 shows, the samples of the several soil-separate fractions were chosen, not equal in weight but such that each contained the same amount of exchangeable calcium, namely, 100 m.e. Yet the apparent surface area of the 13.06 gm. of the clay sample used for each culture was undoubtedly many times that of the sand-separate sample, which weighed 18.38 gm. When the sand-separate particles were mixed with 1,900 gm. of white quartz sand, the average distance between the calcium-bearing particles was relatively great—many times greater than the oscillation distance of the exchangeable ion. Hence, only a relatively minute amount of surface of these calcium-bearing sand particles could have been in actual contact with the plant roots.

The exchangeable ions very probably, therefore, migrated considerable distances through the inert substrate before being absorbed by the roots. It would appear that, in these cultures at least, contact exchange was not the principal mechanism of calcium absorption.

It is possible, of course, that the surfaces of the calcium-bearing sand particles which have lost calcium ions can be replenished by exchangeable ions from within the sand particle. Some of the exchangeable ions of these large calcium-bearing particles must exist within the particle in crevices or channels, because the exchange capacity cannot be accounted for on the basis of the apparent surface of the particles alone. Yet even though ion transfer may occur from within to the surface of the particle, this would appear inadequate to account for the rather large amount of the exchangeable calcium removed by the plants, which in the case of the sand-separate cultures amounted to an average of approximately 28 per cent of the total exchangeable calcium present.

Many factors other than size, such as chemical composition, degree of weathering, and physical state, are involved in the delivery of cations to plant roots by soil particles. For example, silica sand particles would not function in this capacity to any significant degree. These factors have not been treated in this discussion, yet the foregoing experiment emphasizes the fact that certain soil particles large enough to be classified as sand, may have an important role in the supply of nutrient cations to plants.

SUMMARY

In an experiment to determine the comparative availability to plants of exchangeable calcium held by soil particles of different sizes, samples of sand, silt, and clay fractions mechanically separated from Montalto silt loam were chosen to contain equal amounts of exchangeable calcium and were used with white quartz sand as a substrate for the growth of tomato plants, essential ions other than calcium being supplied in soluble form. The results may be briefly summarized as follows:

Plants grew almost equally well in cultures containing sand-, silt-, and clay-separate fractions.

Plants absorbed calcium held in exchangeable form by sand particles almost as rapidly as from silt and clay particles.

Under conditions of this experiment, the absorption of exchangeable calcium from the sand-separate particles apparently occurred largely by processes other than contact exchange.

Particles in the soil as large as those classed as sand cannot be neglected as an important source of available calcium for plants.

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THE IMPORTANCE OF OXYGEN IN THE NUTRIENT SUBSTRATE FOR PLANTS—ION ABSORPTION[1]

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The great determinative effect of the dissolved oxygen tension in the nutrient substrate on the absorption of the nutrient ions by plants has been pointed out by many investigators. Some of the most important work along these lines was carried out by Steward and Preston (12), with potato discs in single-salt or two-salt solutions, and by Hoagland and Broyer (8) and by Burstrom (5) with decapitated roots.

It is, therefore, of great interest and importance to investigate the influence of the dissolved oxygen tensions of a nutrient substrate on the absorption of essential ions from a balanced solution using vigorous intact plants as indicators.

Since the influence of variations in the dissolved oxygen tensions on the absorption of nitrate and ammonium ions by intact plants has been studied by Gilbert and Shive (6) and by Arrington and Shive (2), the present investigation was restricted to a consideration of the calcium, potassium, and phosphate ions.

A number of experimental difficulties presented themselves. Since the rate of absorption of cultures subjected to variations in dissolved oxygen tension varies, the concentrations of nutrients and the pH of the substrates fail to remain constant during the experimental interval, thus introducing variables which affect the quantitative measurements of absorption rates. Furthermore, the use of the continuous flow solution culture method makes it difficult and unwieldy to collect quantitatively all the solution passing by the roots in a given time interval and to determine by analysis of the nutrient solution the relatively small quantity of ions absorbed by the plant. To transfer the plants to fresh solutions every 24 hours means exposing the moist roots to the atmosphere. Some of the oxygen in the air is immediately dissolved in the water films around the roots. This obviously is a source of contamination which should be avoided, since it renders impossible the maintenance of a designated oxygen concentration which, at best, is only an approximation, especially at the low oxygen tensions.

An experimental method was accordingly evolved, which, if it does not completely obviate the aforementioned sources of experimental difficulty, does render them less critical.

MATERIALS AND METHODS

Experimental methods

The apparatus is illustrated in figure 1. The culture vessel B is a 400-ml. beaker, which is calibrated at 350 ml. The beaker is fitted with a rubber stopper containing short lengths of glass tubing through which the plants are inserted,

essentially as described by Gilbert and Shive (6). Through the rubber stopper are also inserted A, a capillary tube by means of which the oxygen mixture is bubbled through the nutrient solution; C, a siphon through which the nutrient solution is removed; and D, a length of glass tubing which is connected by rubber tubing S to the constant-level reservoir R. R consists of a 2-quart Mason jar filled with distilled water inverted in a glass "nappy." A notch is cut into the rim of the Mason jar to allow the glass tube T to be inserted as diagramed.

The constant level reservoir R functions as follows: The apparatus is adjusted so that the solution surface in the "nappy" and the calibration mark on the beaker are at the same level, as indicated by the distance X in figure 1. Thus, when $D\ S\ T$ is filled with water, no movement of water from T to D can occur. Now, as the plants remove water from B through transpiration, the level of the nutrient solution falls in B below the level X and water immediately siphons
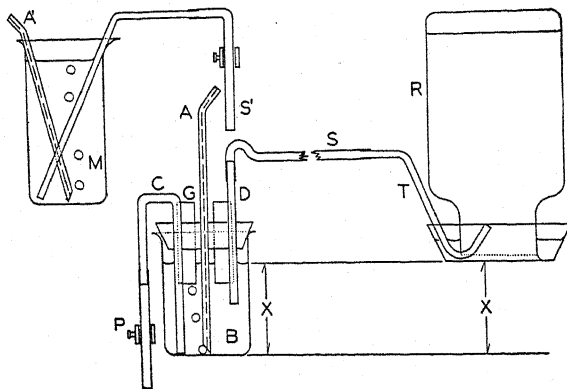
FIG. 1. DIAGRAM OF CULTURE APPARATUS
For explanation see text

over to B from R, restoring the original solution level. By this simple means, a constant volume of solution is maintained in the culture vessel at all times, without regard to the rate of transpiration or other uptake of water by the plants.

M consists of a Berzelius-type liter beaker which is filled with distilled water and brought to the required oxygen level. This is accomplished by passing the proper gas mixture through the capillary aeration tube A'. S' is a simple siphon tube the function of which is explained hereinafter.

The siphon C is used to draw off the nutrient solution for anlysis. It is kept filled with water and closed by a pinch clamp at P.

The culture vessel is surrounded by a light-tight, heavy wrapping paper shield to prevent light from reaching the nutrient solution. These precautions are necessary in order to suppress the growth of algae.

The composition of the nutrient solution used is given in table 1. The stock solution was made up to 3.5 times its normal strength, so that 100 ml. of the

solution could be pipetted into the culture beakers and distilled water added to the calibration mark.   This resulted in a nutrient solution of the proper concentration at exactly the same volume in each culture vessel, without the necessity of disturbing the plants in any way.

The procedure of running an absorption determination may be described stepwise as follows, and for clarity, it will be assumed that an absorption interval has just been concluded:

1. The rubber tubing S is pinched off so as to maintain the siphon, after which it is disconnected from tube D and replaced with S'.

2. Tube P is opened, and the solution is collected in a liter volumetric flask.

3. Just as the last bit of solution is siphoned over, a small portion of water from M is added so as to wash out solution from B.   The additions must be so timed that the siphon at C is not broken.

TABLE 1

Composition of nutrient solution

SALT	CONCENTRATION OF SALT	QUANTITY OF 0.5 M STOCK PER 19 LITERS	ELEMENT	CONCENTRATION OF ELEMENT
	M	$cc.$		$p.p.m.$
Ca(NO$_3$)$_2$..............	0.0047	180.0	Ca	189.5
			N	132.6
KH$_2$PO$_4$...............	0.0012	45.0	K	92.4
			P	36.7
K$_2$SO$_4$.................	0.0012	22.5	S	150.8
MgSO$_4$.................	0.0024	90.0	Mg	56.8
FeSO$_4$.................			Fe	0.50
MnSO$_4$.................			Mn	0.15
H$_2$BO$_3$.................			B	0.25

4. Tube P is then pinched off and from M, B is filled with distilled water, which is then siphoned off, all the wash water being caught in the liter flask.   This procedure effectively washes out all the nutrient solution as well as the material adhering to the roots.

As the level of the solution drops in D, the back pressure on the gases passing through A is decreased, and as a consequence the gas flows more rapidly.   This helps to maintain the proper concentration of oxygen around the moist roots by displacing any atmospheric air which might enter.   The constancy of the oxygen tensions is further maintained by using wash water from M, which has been brought to the proper dissolved oxygen concentration beforehand.   It is admitted that the designated oxygen concentration is not absolutely maintained, but a close approximation to the desired oxygen levels results from this procedure.

5. B is now half filled with water from M, and 100 ml. of the stock nutrient solution is added, either through a tubulature in the rubber stopper, or through the space between the lip of the beaker and the rubber stopper.

6. More water is added from M until the level in B stands slightly below the calibration mark.

7. S' is then removed and S reattached.   The siphon is started by raising R and opening the pinch clamp on S.

8. R is replaced at height X, and the solution in B automatically adjusts itself to the proper level.

9. The solution in the volumetric flask is made up to volume, and aliquots are taken for analysis. The original concentration of the nutrient solution is equal to 100 ml. of stock solution diluted to 1 liter. The milligrams of the element absorbed can then be easily calculated by difference.

It must be emphasized that the various steps should be performed as rapidly as possible in order to minimize the time during which the roots are not in contact with solution. The shorter this time interval is, and the faster the gas flow through A, as the solution in B is removed, the more constant will be the oxygen concentrations around the roots.

The gases used to provide the 4- and 16-p.p.m. treatments were mixtures of oxygen and nitrogen contained in gas cylinders. These mixtures were prepared at the factory from pure water-pumped oxygen and nitrogen. A mixture of 10 per cent oxygen and 90 per cent nitrogen passed through the culture solution at a regulated rate of flow produced an oxygen tension of 4 p.p.m. in the solution, whereas 40 per cent oxygen and 60 per cent nitrogen produced an oxygen tension of 16 p.p.m. Passage of pure nitrogen gas through the solution resulted in 0 p.p.m. Air compressed directly from the atmosphere provided a source of 20 per cent oxygen, which produced a dissolved oxygen tension of 8 p.p.m. when bubbled through the nutrient solutions at a regulated rate of flow.

As shown by Gilbert and Shive (6), and here confirmed, it was possible to maintain the oxygen concentrations only within maximum variations of 25 per cent of the specified values because of oxygen solubility variations with temperature and because of the variations in the rates of oxygen absorption by large, rapidly growing plants. Frequent checks, however, on the dissolved oxygen concentration by means of the Van Slyke manometric apparatus (9), and the subsequent adjustment of the rate of flow of the gases, provided a measure of control. Thus, the maximum variation of 25 per cent of the specified values did not occur often, and the average range of variation of the oxygen tensions from the specified values, over an experimental interval, was relatively narrow.

Analytical methods

Calcium was precipitated as the oxalate and titrated with 0.01 N potassium permanganate essentially as described in the "Official Methods of the A.O.A.C." (3, p. 105). An innovation was introduced, however, by the use of fritted glass filter sticks (Corning #39535, diameter 10 mm., porosity F), in filtering and washing the precipitate. The use of the filter sticks decreased the number of steps in the determination, and as a result, the length of time consumed in each determination was considerably reduced.

Briefly, the filter sticks were used as follows: The calcium oxalate was precipitated in 50-cc. beakers. After the precipitate had been digested and cooled, the supernatant liquid was drawn off by means of the filter stick. The precipitate was washed with successive portions of very dilute ammonium hydroxide solution. Then 5 cc. of 1–4 H_2SO_4 and 10 ml. of distilled water were added, heated to 90°, and titrated with the standard permanganate. All of the pre-

cipitate was effectively dissolved off the filter stick, and no loss was occasioned. The filter stick was subsequently used as a stirring rod during the titration.

Potassium was determined by the method of Hibbard and Stout (7). Immersion filter sticks were also employed as described above, rather than the talc filters described in the original article (7). The precipitate was washed with dilute nitric acid rather than with acetic acid and distilled water, as suggested by Hibbard and Stout.

Phosphorus was determined by the amidol method described by Allen (1), and the color determined by the use of a photoelectric colorimeter.

Experimental plan

The experimental plants were started in sand with a dilute (1–4 standard concentration) nutrient solution. When the first leaves were apparent, uniform seedlings were selected and gently inserted, root first, through the bore of the glass tube supports (G, fig. 1). Each culture contained six plants. A number of extra cultures were set up so that a series of uniform cultures could be selected for the actual experiment. The cultures were supplied with half-strength nutrient solution until they reached the desired stage of growth. They were then placed on a distilled water substrate for 24 hours so as to produce plants of a low salt content by allowing growth to occur, thus decreasing the salt concentration within the tissue. This procedure produced what may be considered a "salt deficit" in relation to growth.

Uniform cultures were selected, placed in a full-strength nutrient substrate, and supplied with the solution at the desired oxygen levels. The absorption of the nutrient elements was determined every 24 hours for 6 days (except series III), according to the procedure outlined above. The absorption intervals in series III were 48 hours, and an experimental interval of 8 days was employed. At the end of the experimental period the plants were removed from the culture vessels, and the roots were washed thoroughly with distilled water, harvested, and dried at 70° for 48 hours. The tissue was weighed, ground in a small Wiley mill, and stored for analysis.

In all the experiments described, young 30-day-old plants were utilized so that the data obtained would be related to as great a quantity of active meristematic tissue as possible. The data resulting from such experiments will reflect the activity of the protoplasmic complex and will not be clouded by the presence of an excessively large amount of dead or metabolically inactive tissue.

Furthermore, short experimental intervals were used so that the experimental plants were all of the same approximate physiological age and growth status. Thus, according to Petrie (10), ontogenetic changes are reduced to a minimum, and the observed effects are truly metabolic in nature.

The experimental plants utilized in the ion-absorption series were tomatoes and soybeans, two species that have been observed to respond differently to variations in the oxygen tensions in the substrate. The experiments were repeated three times with tomato, as the experimental plant, and once with soybeans.

EXPERIMENTAL RESULTS

Influence of dissolved oxygen concentration

Series I—Soybeans (var. Harbinsoy). The daily absorption values for calcium, potassium, and phosphorus at each oxygen level employed for a 6-day experimental period with 30-day-old plants are presented in table 2. The data are expressed in milligrams absorbed per culture (6 plants) per 24 hours. The totals are the sums of the daily absorptions and indicate the total milligrams of the nutrient ions absorbed during the entire experimental period.

It will be observed from the data that the absorption of all the nutrient ions is directly related to the oxygen tension of the substrate. The absorption is least

TABLE 2

Absorption of Ca, P, and K by soybean plants at different oxygen tensions in the nutrient substrate–series I

	CALCIUM				POTASSIUM				PHOSPHORUS			
O₂.........p.p.m.	0	4	8	16	0	4	8	16	0	4	8	16
days	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8.4	11.6	11.6	13.2	28.9	28.9	28.9	28.9	7.6	9.3	10.7	11.4
2	7.0	9.2	10.2	28.0	26.1	28.1	28.3	28.9	7.0	8.4	10.5	12.2
3	9.0	13.2	13.6	6.2	36.9	45.8	52.2	52.2	7.5	8.8	9.5	11.2
4	9.2	8.6	10.0	9.4	35.5	36.8	35.8	36.0	8.6	9.2	11.3	11.5
5	4.6	5.2	5.2	6.6	23.8	19.7	27.0	25.6	6.1	6.2	10.0	9.0
6	1.0	9.8	11.0	8.0	40.7	45.6	50.3	52.7	7.9	8.3	11.7	12.0
Total......	39.2	57.6	61.6	71.4	191.9	204.9	222.5	224.3	44.7	50.2	63.7	67.3
Average per plant.......	6.5	9.6	10.3	11.9	32.0	34.1	37.1	37.4	7.4	8.4	10.6	11.2
Ratio*.......	1.0	1.5	1.6	1.8	1.0	1.1	1.2	1.2	1.0	1.1	1.4	1.5

* Absorption rates at different oxygen tensions based on absorption rate at 0 p.p.m. as unity.

at 0 p.p.m. and increases with increasing oxygen tension to the maximum at 16 p.p.m. The total values emphasize this point. It is evident, therefore, that the metabolic activity of the roots, as controlled by the oxygen tension of the substrate, has a great determinative effect upon the absorption of ions by the roots. Low metabolic activity, which is produced by semianaerobic conditions resulting from deficient oxygen, retards absorption rates, whereas an increasing available dissolved oxygen supply, approaching the optimum level, produces an aerobic condition and a concommittant increase in the absorption rate of the nutrient ion.

Series II—Tomato (var. Marglobe). Table 3 presents a similar set of data obtained by using the tomato as the experimental plant. Again, the absorption rate bears a direct relation to the dissolved oxygen tension. In this case, however, 8 p.p.m. of dissolved oxygen corresponds to the maximum absorption

rate, and 16 p.p.m. retards the absorption rate to a point considerably below the optimum. This decrease in the absorption rates at 16 p.p.m. proved to be a

TABLE 3

Absorption of Ca, P, and K by tomato plants at different oxygen tensions in the nutrient substrate—series II

O₂.........p.p.m.	CALCIUM				POTASSIUM				PHOSPHORUS			
	0	4	8	16	0	4	8	16	0	4	8	16
days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	10.4	10.8	11.8	15.0	11.5	15.0	29.7	22.6	3.2	4.2	3.5	3.5
2	12.2	12.8	16.8	13.4	20.3	26.4	31.1	31.2	3.2	4.2	14.5	5.9
3	9.6	9.4	60.0	28.8	21.6	28.1	31.2	31.1	4.3	5.6	5.9	8.0
4	12.2	9.6	32.4	21.0	20.0	25.6	31.8	31.9	4.9	6.4	8.3	7.9
5	13.4	14.4	17.6	19.0	31.4	31.8	31.2	31.8	6.9	9.0	10.0	8.9
6	9.6	12.2	23.6	26.4	31.2	31.8	30.8	31.8	5.7	7.4	8.0	9.7
Total......	67.4	69.2	162.2	123.6	136.0	158.7	185.8	180.4	28.2	36.8	50.2	43.9
Average per plant.......	11.2	11.5	27.0	20.6	22.7	26.5	30.9	30.1	4.7	6.1	8.4	7.3
Ratio*.......	1.0	1.0	2.4	1.8	1.0	1.2	1.4	1.3	1.0	1.3	1.9	1.6

* Absorption rates at different oxygen tensions based on absorption rate at 0 p.p.m. as unity.

TABLE 4

Absorption of Ca, P, and K by tomato plants at different oxygen tensions in the nutrient substrate—series III

O₂..............p.p.m.	CALCIUM			POTASSIUM			PHOSPHORUS		
	0	8	16	0	8	16	0	8	16
days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
2	24.0	57.6	34.8	17.2	18.5	18.0	2.2	6.0	3.0
4	18.8	33.6	25.6	16.0	17.2	17.8	1.4	3.4	2.8
6	96.4	107.6	102.0	16.5	17.2	17.6	5.4	14.5	7.3
8	26.0	62.4	47.6	15.5	17.5	17.0	2.2	12.8	6.5
Total...........	165.2	261.2	21.00	65.2	70.4	70.4	11.2	36.7	19.6
Average of four plants...........	41.3	65.3	52.5	16.3	17.6	17.6	2.8	9.2	4.9
Ratio*............	1.0	1.6	1.3	1.0	1.1	1.1	1.0	3.3	1.8

* Absorption rates at different oxygen tensions based on absorption rate at 0 p.p.m. as unity.

constant characteristic of tomato plants. Oats, in a preliminary experiment, not reported here, showed a similar falling off in absorption rates at 16 p.p.m. of oxygen in the substrate.

The calculated ratios in the last line of table 1 and of table 2 indicate a very important fact, which would not be discerned readily from the recorded data of the tables. It will be observed that calcium and phosphorus are influenced to a greater extent by the differences in oxygen tensions than is potassium. These relationships are more clearly defined in the next series.

Series III—Tomato (var. Marglobe). In series III the determinations of ion absorption were made every 48 hours. This prolonged absorption interval allowed greater differences to become apparent. In addition, the environmental conditions during this experimental interval were somewhat less erratic with respect to temperature, light, and humidity than in series I and II. Only three oxygen levels were used.

Table 4 gives the data obtained. It will be observed that the same trends as in series I and II were obtained. The optimum absorption rates are again attained at 8 p.p.m. of oxygen, and again, at 16 p.p.m. of oxygen in the substrate, they fall below the optimum.

Behavior of individual ions

The influence of the dissolved oxygen tensions in the substrate on the absorption rates of the various nutrient ions investigated is not the same. This fact is illustrated in figures 2, 3, and 4. The ordinates represent the average milligrams of the element in question absorbed from the nutrient solution per plant. These are plotted against time as the abscissas. The individual points on the curve are summation points. That is to say, they represent the total amount of the element in question absorbed from the start of the experimental period to the time in days indicated. The slope between any two points represents, therefore, a basis for the measure of the rate of absorption during that time interval, and the distance along the ordinate between the individual curves represents the effect of the dissolved oxygen tension on the rates of absorption.

Figure 2, represents the absorption of calcium by the plants in series III plotted against time as above described. The great dependency of absorption rates upon the oxygen tension of the substrate is evident from the spacing of the graphs. The points on the curve representing absorption at an oxygen level of 8 p.p.m. are separated by relatively large values from those on the curve of absorption at the oxygen level of 0 p.p.m. The retarding effect of the high oxygen level (16 p.p.m.) on the absorption of calcium by tomato plants is indicated by the fact that the curve of absorption at this oxygen level lies between those at 0 p.p.m. and 8 p.p.m.

Figure 3, indicates the effect of oxygen tensions employed on the absorption of phosphorus. Here again, there is shown a direct dependency of the absorption of phosphorus on the oxygen concentrations supplied to the roots. The retarding effect of excessive oxygen tension on absorption is again demonstrated by the fact that the graph representing phosphorus absorption at the highest oxygen level lies intermediate between the other two graphs.

Figure 4, which presents the absorption rate of potassium at the several oxygen tensions, plotted against time, demonstrates a very different absorption pattern

from that indicated for calcium and phosphorus with the tomato seedlings as the indicator plant.

These graphs, representing potassium absorption at each of the three oxygen levels over a total period of 8 days, indicate that the removal of potassium from the substrate by the plant is a straight-line function of time. Since two of the graphs are virtually superimposed, and since the third (absorption at 0 p.p.m. oxygen) is only slightly below the other two, they clearly indicate that the
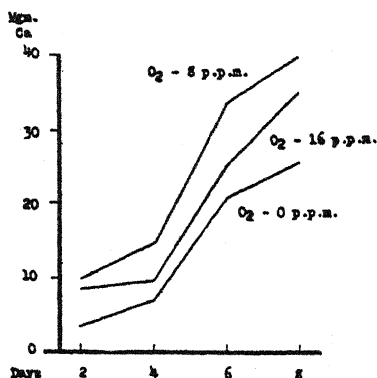

FIG. 2. ABSORPTION OF CALCIUM BY TOMATO PLANTS IN MILLIGRAMS PER 48 HOURS PER PLANT AT DIFFERENT OXYGEN CONCENTRATIONS IN THE SUBSTRATE
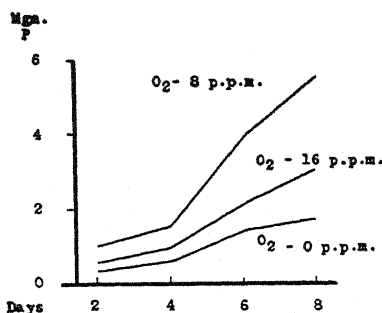

FIG. 3. ABSORPTION OF PHOSPHORUS BY TOMATO PLANTS PER 48 HOURS PER PLANT AT DIFFERENT OXYGEN CONCENTRATIONS IN THE SUBSTRATE

absorption of potassium by the tomato plant is not so materially influenced by the oxygen levels of the substrate, under the experimental conditions here considered, as are calcium and phosphorus. In this, potassium absorption differs notably from the absorption of the other ions, which is, in large measure, determined by the oxygen level of the substrate. This is borne out also by the ratio figures of table 2 and 3.

The explanation of these observations may be associated with the fact that of all the nutrient ions, potassium is the least hydrated, is completely soluble within the plants, and possesses, therefore, a high degree of mobility. It is

possible that because of these properties, absorption of potassium falls less than does that of the other ions, under the control of respiratory processes, which are dominated by the oxygen supply in the substrate.

There is, nevertheless, even for the potassium ion, a definite relationship between deficient aeration and inadequate nutrient uptake, as is indicated by the data of tables 2, 3, and 4. Other workers (4, 12), have obtained similar results, and it appears certain that absorption of all cations and anions is a direct function of the metabolic activity of the roots, and that any explanation of ion accumulation purely on the basis of diffusion phenomena is inadequate.
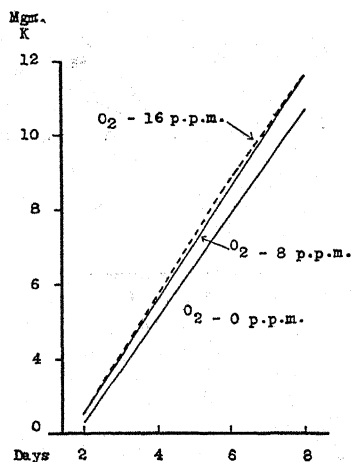

FIG. 4. ABSORPTION OF POTASSIUM BY TOMATO PLANTS IN MILLIGRAMS PER 48 HOURS PER PLANT AT DIFFERENT OXYGEN CONCENTRATIONS IN THE SUBSTRATE

Influence of climatic conditions

The absorption of various nutrient ions by the roots was further influenced by the activity of the tops. Although the experiments described were not designed toward this end, the data obtained demonstrate some aspect of the influence of the tops on absorption by the roots.

It will be observed from tables 2, 3, and 4 that the absorption rates varied considerably from day to day. These variations were observed to depend upon the climatic conditions during the absorption intervals in question. Absorption rates during a clear, bright day, with low humidity, always were higher than those during an interval characterized by low light intensity and high humidity, as under an overcast sky. From these observations it appears that the high transpiration rates and the general vigorous activity of the plants on clear, dry days resulted in a greater absorption of nutrient ions, especially calcium and phosphorus, than when the transpiration rates were generally low. Here temperature also was a factor that contributed a share in the general activity of the plant and its processes. However, the general activities of the plant,

whatever may be the factors which control them, appeared to have much less influence on the rates of potassium absorption, as indicated by the linear relationship to time, than on those of any of the other ions here considered.

DISCUSSION

Aside from the demonstrated fact that there was a direct relationship between aerobic respiration and ion absorption and accumulation, a fact which has been substantiated by several investigators (2, 4, 8), there is little knowledge concerning the actual mechanism involved in the absorption of ions. It is manifestly impossible, therefore, to point out the exact function of the dissolved oxygen in the absorption mechanism other than to say that it has a determinative influence upon respiration, which in turn has a regulatory influence in maintaining the metabolic status of the cells, including the absorption mechanism.

Gilbert and Shive (6) have shown that plants can absorb nitrates at a higher rate under conditions of low oxygen tension in the nutrient substrate than when the dissolved oxygen supply is adequate for normal growth and development. This is due to the fact that when the supply of oxygen for respiration is deficient, the plants rapidly reduce the absorbed nitrate ions, and in the process, oxygen is released and becomes available for the respiratory processes. It is interesting to consider whether oxygen could be released from oxygen-containing complexes, other than nitrate ions, which could be utilized in the respiratory processes. Theoretically, the absorption of the phosphate ions should not yield active oxygen which could be utilized in respiratory processes, since this ion enters into combination in the organic complexes directly as the phosphate and does not undergo reduction in the same manner as does the nitrate ion.

The data presented show that the absorption of the phosphate ion was directly influenced by the oxygen tension of the substrate, that is, low rates of absorption of the phosphate ion were directly associated with low oxygen levels, and high rates of absorption, with high oxygen levels. It is thus evident that absorbed phosphate ions, unlike absorbed nitrate ions, cannot provide a source of active oxygen which may be utilized in the respiratory processes under anaerobic conditions. This is in accord with theoretical considerations.

On the other hand, reduced sulfur groups do occur in the plants. It is conceivable that the reduction of the sulfate ion might release active oxygen which might function in respiration, under anerobic conditions. The quantity of active oxygen which might be supplied from this source would, of necessity, however, be too small to be materially effective in anaerobic respiration. The data obtained in the present investigation provide no evidence bearing upon this question.

SUMMARY

A culture apparatus and experimental method have been presented for the direct determination of ion absorption by plants at approximately maintained dissolved oxygen tensions in the substrate by periodic analysis of the nutrient solution without disturbing the plants.

The tomato and the soybean were used as the indicator plants, and the daily

absorption of potassium, calcium, and phosphorus was determined over weekly experimental intervals at oxygen levels of 0, 4, 8, and 16 p.p.m.

The absorption of all the nutrient ions considered was directly related to the oxygen tension of the substrate. The absorption of the nutrient ions was least at 0 p.p.m. and rose to an optimum at the higher oxygen levels.

For soybeans, the maximum absorption rate was obtained at 16 p.p.m.

The maximum absorption for tomatoes was at 8 p.p.m. In the case of these plants, 16 p.p.m. retarded the absorption rate to a point below the optimum.

The absorption of calcium and phosphorus was directly dependent upon the dissolved oxygen supply. Potassium, however, was not so materially influenced by the oxygen levels of the substrate but demonstrated a greater independence than did calcium and phosphorus in relation to the various oxygen levels.

The data indicated that the generally vigorous activity of the plants on clear, dry days accelerated the absorption of nutrient ions, especially calcium and phosphorus. These factors, however, have much less influence in modifying the rates of potassium absorption than they do those of any of the other ions here considered.

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THE SYNTHESIS OF LIGNIN-LIKE COMPLEXES BY FUNGI

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The ability of some of the common soil fungi to synthesize lignin-like complexes when grown on a sucrose-nitrate medium was demonstrated by Thom and Phillips (8). The black and brown species, commonly found growing on decaying vegetation at the soil surface, contained up to 29 per cent of these lignin-like substances, whereas the colorless or light-colored fungi contained little lignin. Likewise, some of the brown and black wood-destroying bracket fungi contained as much as 54 per cent of these materials, whereas the light-colored species contained only about 2 to 4 per cent. In a later paper Phillips (5) reported a chemical study of the lignin-like material in one of these bracket fungi. This material was found to resemble lignin in some of its chemical reactions but contained almost no methoxyl groups. Although Thom and Phillips (8) pointed out the possible relationship of these lignin-like substances in the filamentous fungi to the formation of soil organic matter, no further work along these lines has been reported. The present paper presents results obtained with 12 filamentous fungi, mostly species that are found in the soil or on decaying plant materials. Both pigmented and unpigmented species were selected.

METHODS

The fungi were grown in 3-liter wide-mouth Erlenmeyer flasks stoppered with cotton and containing 500 ml. of nutrient medium.

The compositions of the media used, apart from the nitrogen source, were as follows:

A. Czapek's medium: K_2HPO_4, 1.0 gm.; KCl, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; $FeSO_4 \cdot 7H_2O$, 0.01 gm.; sucrose, 30 gm.; and distilled H_2O, 1 liter.

B. Same as A except NaCl, 0.2 gm., instead of KCl, 0.5 gm., and trace elements instead of $FeSO_4 \cdot 7H_2O$, 0.01 gm.; plus $CaSO_4 \cdot 2H_2O$, 0.1 gm.

C. Same as B plus $CaCO_3$, 10 gm.

D. Same as B except K_3PO_4, 1 gm., and K_2HPO_4, 0.5 gm., instead of K_2HPO_4, 1 gm.

The trace elements added to media B, C, and D were supplied at the rate of 10 ml. per liter of a solution having the following composition: $FeSO_4 \cdot 7H_2O$, 150 mgm.; $ZnSO_4 \cdot 7H_2O$, 125 mgm.; $CuSO_4 \cdot 5H_2O$, 30 mgm.; $MnCl_2 \cdot 4H_2O$, 25 mgm.; $NaMoO_4 \cdot 2H_2O$, 4.5 mgm.; and distilled H_2O, 1 liter.

The media were sterilized in the autoclave, inoculated in duplicate or triplicate with pure cultures, and incubated at 25°C. for 1 month.

Prior to deciding upon the temperature of incubation to be used, a preliminary experiment was run with four cultures grown at both 25° and 30°C. The rates of growth of *Cladosporium fulvum* 5668, *Cladosporium* 5464.2, and *Alternaria* were about equally good at the two temperatures, whereas *Helminthosporium* 5428.16 grew somewhat better at the lower temperature. The weight of fungus obtained per unit of sugar consumed was about 20 per cent higher at the lower temperature.

At the end of the incubation period the pH values of the media were determined colorimetrically and the sugar contents by the method of Shaffer and Hartmann (7). The fungus mat was dried at 40 to 45°C. for about 5 days to constant weight. This method was used to avoid decomposition, which was observed to take place at 105°C., and also to minimize errors in the determination (3) of lignin.

Lignin-like material was determined by the 72 per cent sulfuric acid method (6), and the corrected value was obtained by subtracting the protein (nitrogen × 6.25). The protein correction in this particular study is only an approximation, since fungi may contain varying amounts of nonprotein nitrogenous substances. The ash content of the lignin fractions of three of the fungi varied only from 0.5 to 0.76 per cent; hence this determination was not made on the remainder of the samples. The slight correction was ignored in reporting the results.

<div align="center">EXPERIMENTAL RESULTS</div>

The pertinent data concerning the growth and carbohydrate consumption of the various species of fungi are given in table 1 and the analyses of the fungus mats in table 2. All of the data in the two tables are average values of either duplicate or triplicate cultures.

In general, all of the organisms studied made fairly good growths under the conditions of culture, provided the reaction of the medium did not become appreciably acid. The growths obtained in the moderately acid media were lower than expected. Although these fungi tolerated acid media it is quite apparent that they did not thrive under these conditions. In most cases where the media were kept at near neutrality the sugar was virtually all consumed during the 30-day growth period.

All three sources of nitrogen were utilized by the six cultures of fungi used in the comparative tests. In most cases sodium nitrate gave better results than ammonium sulfate or ammonium nitrate, chiefly because it kept the pH at a higher level. Where pH was not a factor there were usually no marked differences between the three nitrogen sources; in fact, with some of the organisms ammonia nitrogen gave higher yields than did nitrate.

The most satisfactory of the four media was C, containing trace elements and adequate $CaCO_3$ to keep the reaction above the neutral point. It is evident that the trace elements, and calcium, favored growth, although there was no critical comparison of growth with and without these elements in media otherwise identical.

TABLE 1

Growth and efficiency of sugar utilization of several fungi grown in different media
Incubated 30 days at 25°C.

FUNGUS	MEDIUM USED	NITROGEN SOURCE*	pH OF MEDIUM	WEIGHT OF FUNGUS	SUGAR CONSUMED	ECONOMIC COEFFICIENT†	CARBON ASSIMILATION‡
				gm.	*gm.*	*per cent*	*per cent*
Cladosporium 5464.2	A	NaNO$_3$	8.0	5.097	15.00	34.0	42.4
	B	NaNO$_3$	8.2	5.207	15.00	34.7	43.2
	B	(NH$_4$)$_2$SO$_4$	2.4	1.606	6.05	26.6	33.2
	B	NH$_4$NO$_3$	2.4	1.920	7.25	26.5	33.1
	C	NaNO$_3$	8.4	5.027	15.00	33.6	41.9
	C	(NH$_4$)$_2$SO$_4$	7.3	5.869	14.76	39.7	49.6
	C	NH$_4$NO$_3$	7.6	5.392	14.84	36.4	45.4
	D	NaNO$_3$	7.4	3.034	11.12	27.3	34.1
	D	(NH$_4$)$_2$SO$_4$	5.4	0.398	1.75	22.9	28.6
	D	NH$_4$NO$_3$	5.4	0.647	2.73	23.8	29.7
Cladosporium fulvum 5668	A	NaNO$_3$	6.4	1.421	8.11	17.5	20.7
	B	NaNO$_3$	8.1	5.033	15.00	33.5	39.6
	B	(NH$_4$)$_2$SO$_4$	2.0	2.249	10.40	21.3	25.2
	B	NH$_4$NO$_3$	6.8	5.305	14.89	35.6	42.0
	C	NaNO$_3$	7.8	5.344	14.80	36.1	42.6
	C	(NH$_4$)$_2$SO$_4$	7.2	5.875	14.75	39.8	47.0
	C	NH$_4$NO$_3$	7.6	5.586	15.00	37.2	43.9
	D	NaNO$_3$	8.2	5.293	15.00	35.3	41.7
	D	(NH$_4$)$_2$SO$_4$	4.4	2.126	10.49	20.2	23.9
	D	NH$_4$NO$_3$	7.2	5.568	14.59	38.1	45.0
Helminthosporium 5428.16	A	NaNO$_3$	7.9	3.123	15.00	20.8	24.4
	B	NaNO$_3$	8.2	4.543	15.00	30.2	35.4
	B	(NH$_4$)$_2$SO$_4$	2.3	0.970	4.41	21.9	25.6
	B	NH$_4$NO$_3$	2.3	1.010	4.96	20.3	23.8
	C	NaNO$_3$	8.6	4.179	15.00	27.9	32.7
	C	(NH$_4$)$_2$SO$_4$	7.6	5.016	15.00	33.4	39.1
	C	NH$_4$NO$_3$	8.0	5.184	15.00	34.6	40.5
	D	NaNO$_3$	8.4	4.423	15.00	29.5	34.5
	D	(NH$_4$)$_2$SO$_4$	4.5	1.470	6.70	21.9	25.6
	D	NH$_4$NO$_3$	7.2	4.960	15.00	33.1	38.8
Alternaria	A	NaNO$_3$	7.5	1.505	7.58	19.7	21.7
	B	NaNO$_3$	7.7	2.892	13.19	21.9	24.1
	B	(NH$_4$)$_2$SO$_4$	2.4	0.481	3.14	15.3	16.8
	B	NH$_4$NO$_3$	3.0	1.548	5.78	26.8	29.5
	C	NaNO$_3$	7.7	3.438	14.16	24.2	26.6
	C	(NH$_4$)$_2$SO$_4$	7.9	0.607	3.68	17.0	18.7
	C	NH$_4$NO$_3$	7.9	3.778	14.73	25.7	28.3
	D	NaNO$_3$	7.6	2.823	13.62	20.6	22.7
	D	(NH$_4$)$_2$SO$_4$	2.4	0.678	3.48	19.5	21.5
	D	NH$_4$NO$_3$	6.0	2.632	9.50	27.8	30.6

TABLE 1—*Concluded*

FUNGUS	MEDIUM USED	NITROGEN SOURCE*	pH OF MEDIUM	WEIGHT OF FUNGUS	SUGAR CON-SUMED	ECONOMIC COEFFI-CIENT	CARBON ASSIMI-LATION
				gm.	*gm.*	*per cent*	*per cent*
Gliocladium fimbriatum	A	NaNO₃	6.6	1.175	15.00	7.8	9.4
459.5128	B	NaNO₃	8.4	4.727	15.00	31.5	37.8
	B	(NH₄)₂SO₄	2.1	1.715	6.05	28.3	34.0
	B	NH₄NO₃	7.6	4.549	15.00	30.4	36.5
	C	NaNO₃	8.4	4.380	15.00	29.2	35.0
	C	(NH₄)₂SO₄	7.5	5.206	15.00	34.7	41.6
	C	NH₄NO₃	7.8	4.549	15.00	30.4	36.5
	D	NaNO₃	8.4	4.866	15.00	32.5	39.0
	D	(NH₄)₂SO₄	4.6	3.043	11.63	26.4	31.7
	D	NH₄NO₃	7.7	4.320	15.00	28.8	34.6
Metarrhizium	A	NaNO₃	6.6	0.299	9.89	3.0	3.2
	B	NaNO₃	8.3	3.289	15.00	21.9	23.7
	B	(NH₄)₂SO₄	2.4	0.927	5.92	15.7	17.0
	B	NH₄NO₃	7.1	3.085	15.00	20.6	22.3
	C	NaNO₃	7.4	4.310	15.00	28.7	31.0
	C	(NH₄)₂SO₄	7.7	2.750	15.00	18.4	19.9
	C	NH₄NO₃	8.4	3.625	15.00	24.2	26.1
	D	NaNO₃	8.6	3.590	15.00	24.0	25.9
	D	(NH₄)₂SO₄	2.4	1.075	5.81	18.6	20.1
	D	NH₄NO₃	7.6	3.153	15.00	21.0	22.7
Dematium pullulans 5695.354D	B	NaNO₃	7.9	3.555	14.80	24.0	25.2
Aspergillus giganteus 5–5581.13A	B	NaNO₃	8.2	3.534	14.80	23.9	24.1
Aspergillus niger 142	B	NaNO₃	7.4	3.672	15.00	24.5	24.7
Gliocladium 454–4640.428	B	NaNO₃	8.6	4.713	15.00	31.4	39.6
Helminthosporium 27.07	B	NaNO₃	8.6	5.528	15.00	36.9	38.4
Humicola 5239	B	NaNO₃	8.2	3.524	15.00	23.5	26.3

* Nitrogen was added at the rate of 3.0 gm. per liter of sodium nitrate or its equivalent.
† Weight of fungus divided by weight of sugar consumed.
‡ Weight of carbon in fungus divided by weight of carbon in sugar utilized.

The efficiency of use of the sugar supplied is commonly expressed in terms of weight of fungus produced divided by weight of sugar consumed (economic coefficient), or weight of carbon in the fungus divided by weight of carbon in the sugar used. The latter value is usually somewhat higher than the former because the percentage of carbon in the fungus is commonly higher than that in sugar. Both values are given in table 1.

Under the best conditions, the percentage of the carbon in the sugar that was converted into cell material was approximately 40 to 50 for *Cladosporium, Helminthosporium,* and *Gliocladium,* and 25 to 30 for *Alternaria, Metarrhizium, Dematium, Aspergillus,* and *Humicola.* These data are in good agreement with values of 30 to 50 per cent reported by Waksman (10, p. 244). In almost all cases where

the growth conditions were poor and a considerable portion of the added sugar was not consumed, less fungal material was formed per unit of sugar consumed than under good growth conditions. Apparently this observation holds for most microorganisms kept under ordinary atmospheric conditions.

The percentage of carbon in the 12 cultures of fungi, given in table 2, varied from 42.7 to 53.1. This compares with a value of 42.1 for sucrose. The nitrogen content varied from 2.4 to 4.4 per cent. This value would, of course, be expected to vary markedly with the quantity of nitrogen supplied. The C:N ratios averaged 15.3 with variations from 10.7 to 22.4. These values are not very much higher than those of the majority of the soils in the northern half of the United States.

The content of crude lignin varied from 2.8 to 30.9 per cent for the various organisms studied. This crude lignin consists of lignin-like complexes, ash, and

TABLE 2

Composition of the fungi studied

FUNGUS	CARBON	NITRO-GEN	C:N RATIO	CRUDE LIGNIN	PROTEIN IN CRUDE LIGNIN	LIGNIN	SUGAR CON-VERTED TO LIGNIN
	per cent	per cent		per cent	per cent	per cent	per cent
Cladosporium 5464.2...............	52.52	2.41	21.8	30.90	6.93	23.97	12.0
Cladosporium fulvum 5668..........	49.75	2.56	19.4	25.05	6.85	18.20	8.9
Helminthosporium 5428.16..........	49.40	3.71	13.3	24.85	8.25	16.60	7.3
Helminthosporium 27.07............	43.92	3.21	13.7	27.65	6.15	21.50	11.5
Aspergillus giganteus, 5–5581.13A..	42.68	3.83	11.1	9.70	3.35	6.35	2.2
Aspergillus niger 142..............	42.70	2.66	16.1	9.50	3.10	6.40	2.3
Gliocladium fimbriatum 459.5128....	50.42	2.90	17.4	3.79	0.93	2.86	1.3
Gliocladium 454–4640.428..........	53.10	2.37	22.4	2.78	0.80	1.98	0.9
Alternaria.........................	46.18	4.03	11.5	9.20	2.40	6.80	2.2
Dematium pullulans 5695.354D.....	44.07	3.59	12.3	10.58	3.48	7.10	2.5
Metarrhizium......................	45.65	3.24	14.1	5.45	1.88	3.57	1.1
Humicola 5239.....................	47.02	4.39	10.7	12.78	4.65	8.13	2.8

some unknown organic nitrogenous materials. The quantities of these last-named materials, expressed as proteins, are shown in the table. By subtracting these values from those given for crude lignin, the percentages of lignin, or lignin-like materials, are obtained. The methoxyl contents of the lignin-like materials in *Cladosporium fulvum* 5668, *Cladosporium* 5464.2, and *Helminthosporium* 5428.16 were 0.65, 1.03, and 0.32 per cent respectively. These values are much lower than those commonly reported for the lignins of higher plants and are in agreement with the findings of Phillips (5).

The last column in table 2 shows the percentage of the carbon in the sugar assimilated that was converted into lignin-like materials. Phillips' figure of 61 per cent carbon in the lignin of fungi was used in making these calculations.

These data confirm the conclusions of Thom and Phillips that the black or brown species of fungi, so far as tested, are able to synthesize considerable quan-

tities of lignin-like materials, whereas the light-colored species synthesize smaller amounts or none. According to the results in table 2 all species synthesized some of these materials, but of course the method used includes any material that resists the sulfuric acid treatment.

DISCUSSION

These studies are of interest from the standpoint of practical agriculture, since they show that certain types of soil fungi, if supplied with adequate mineral nutrients including nitrogen, can convert an energy source, such as sugar, into lignin-like materials. These crude lignin-like materials have C:N ratios and certain other properties similar to those of soil humus. Most soil fungi can utilize a wide variety of sources of energy; even cellulose is converted into lignin by some of the organisms used in the present studies. Work along these lines is now in progress.

Thom and Phillips point out that the black and brown molds thrive on decaying vegetation found at or just above the soil surface and not on the same materials after being plowed under. This fact is of interest in connection with the present tendency to emphasize the merits of keeping the crop residues on the surface, as in stubble mulch farming. In the process of decomposition, whether at the surface or on the plow sole, much of the lignin in the plant residues would resist decomposition and be converted into humus. In addition, if lignin-synthesizing organisms are active, considerable humus should also be formed from the sugar, cellulose, and other readily available carbon sources. It is well, however, not to overemphasize this point until we have practical information to support the laboratory results and indications.

The rate and the completeness of decomposition of fungal tissues of various species have been considered by a number of investigators. The conclusions of the earlier workers differ markedly, as pointed out by Jensen (2). Most of these workers observed that both bacterial and fungal cells decomposed rather readily although a few mentioned the formation of resistant humus-like residues. In Jensen's work no clear influence of C:N ratio of the microbial substance on decomposition was observed. On the other hand, Heck (1) and Norman (4) found a very clear correlation between the C:N ratio of the fungus materials and the rate of decomposition. In many cases Jensen obtained colloidal, brown to black, humus-like compounds as residues from the decomposition of fungi. Waksman (9), likewise, reported the synthesis of similar substances by mixed cultures using cellulose as an energy source. In contrast, Norman says that he found no evidence for the existence of a very resistant residue from fungal tissue. The present studies show that these differences among workers can be explained in large part by the composition of the organisms used. Apparently neither Norman nor Heck used species of fungi that we would now expect to be synthesizers of large quantities of lignin-like compounds. On the other hand, at least one of Jensen's species (*Stachybotrys* sp.) probably possesses this ability. The mycelia of *Cladosporium* and *Helminthosporium*, used in the present study, would probably decompose as did Jensen's *Stachybotrys*, whereas *Gliocladium* would be expected to behave as did the organisms used by Heck and by Norman.

SUMMARY

The synthesis of lignin-like complexes by 12 cultures of filamentous fungi grown on a mineral-sucrose medium was studied. The mycelium of *Cladosporium* contained as high as 24.0 per cent of these lignin-like substances. The average values for the genera studied were *Cladosporium* 21.1, *Helminthosporium* 19.0, *Humicola* 8.1, *Dematium* 7.1, *Alternaria* 6.8, *Aspergillus* 6.4, *Metarrhizium* 3.6, and *Gliocladium* 2.4. These values represent the nonnitrogenous portion of the fungal substance that resisted digestion with 72 per cent sulfuric acid. In general, the black or brown fungi were comparatively high in lignin complexes, whereas the colorless or light-colored organisms contained lower percentages. The failure of previous workers, using various genera, to agree as to the ability of fungi to form substances that are resistant to decay is largely explained by these results.

Under the best growth conditions, that is at pH 7 or slightly above and with adequate trace elements present, approximately 40 to 50 per cent of the carbon of the sucrose was converted into cell material by *Cladosporium*, *Helminthosporium*, and *Gliocladium*; the corresponding values for the other organisms were 25 to 30 per cent. The C:N ratios of the fungus material varied between 10.7 and 22.4 per cent with an average value of 15.3.

It is pointed out that the high-lignin organisms grow mostly on decaying vegetation at or above the soil surface. A system, such as that used in stubble mulch or trash mulch farming, would therefore be expected to be favorable to a high yield of humus.

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BOOKS

Artificial Manures. By ARTHUR B. BEAUMONT. Judd Publishing Company, Inc., New York, 1943. Pp. 155, illus. 38. Price, $1.50.

This book has for its purpose a consideration of the various means by which substitutes for animal manures can be made available for use, the need for this arising out of the replacement of the horse by the automobile and the tractor. The manure problem is considered from the point of view of the home gardener, the market gardener, the truck-crop farmer, and the man who operates large acreages of land. For this large operator, the method is known as "sheet-compositing," a new term for a modified form of green manuring. The illustrations are impressive and give the reader a new concept of the wide scale on which the production of artificial or "synthetic" manure is being developed. The book is worth more than it costs.

Encyclopedia of Substitutes and Synthetics. By MORRIS D. SCHOENGOLD. Philosophical Library, New York, 1943. Pp. 382. Price, $10.

This book gives the properties of a great variety of products that have been developed for or could be used as substitutes for other products not now available for purchase. Examples are Abelmoschus, Atabrine, Fagelanes, lecithin, and Prosein, substitutes for musk, quinine, leather, egg yolk, and casein, respectively. Kaolin and diatomaceous earth are among the more important products of interest to workers in soil science. The author points out that the word "substitute" is a misnomer, inasmuch as the substitute often proves superior to the material it was supposed to replace.

Exploring Tomorrow's Agriculture. By JOSEPH W. EATON. Harper and Brothers, New York, 1943. Pp. 255, illus. 9. Price, $2.75.

This is a report of a study sponsored by the Rural Resettlement Institute. The three parts deal with cooperative group farming as a method of rural rehabilitation, the cooperative corporation farms of the Farm Security Administration, and other cooperative group farms. The FSA had 27 cooperating corporation farms established as of March, 1942, of which about half are in the South, a third in the Middle West, and the remainder in the Far West. Chapters on the utopian farm groups of the past, the "Hutterische Gemein," the Amana Society, and certain of those in foreign countries are appended. The material is constructively presented, and the book merits careful reading by those who have to do with the less productive agricultural areas of this country.

Farm Management. By ROBERT R. HUDELSON. The MacMillan Company, New York, 1943. Pp. 396. Price, $2.50.

This book deals with the organization, planning, operating, and financing of a modern farm. It considers such important problems as choosing the farm, deciding on a cropping system, planning the livestock program, controlling pro-

duction hazards, marketing the products, keeping farm records, and gaining the ownership of the land. If every young man could have this book at his disposal early in his career on the land, and if he would study its contents carefully, he should be able to avoid many of the pitfalls that await those who undertake the business without an adequate understanding of the fundamental principles on which success with the soil is based.

Food Enough. By JOHN D. BLACK. The Jaques Cattell Press, Lancaster, Pennsylvania, 1943. Pp. 269, charts 13. Price, $2.50.

This is one of the "Science for War and Peace Series" of books. In it the author attempts to present the facts upon which all considerations of the food problem should be based. Anyone who desires to speak or write on the food problem would do well to consult the volume. In fact, the author thinks that even the farmer-author, Louis Bromfield, though "skilled with the pen" and having "a chance to blow off before a larger public," has the facts and figures "about the food program about as accurately in mind" as his barber and his dentist. Even the county agents, for whom the author has great respect, err seriously in their judgment of conditions in their respective counties, for "The only farmers who bother the county agent about farm labor are those who are short of labor," and his report will be "twice what has occurred." Food, manpower, machinery, prices, rationing, relief, rehabilitation, and after-the-war problems are all considered in a very readable manner.

The Food Resources of Africa. By THOMAS S. GITHENS AND CARROLL E. WOOD, JR. The University of Pennsylvania Press, Philadelphia, 1943. Pp. 105, figs. 24. Price, $1.50.

This is the third of a series of eight African handbooks dealing with the government, mineral resources, food resources, languages, colonial policies, and labor problems of Africa. It is apparent from the contents of this book that the agricultural potentialities of Africa are very large but that they cannot be realized because of the primitive practices of the natives. Of greatest interest at the moment is the better organized agriculture of North Africa. Thus the area devoted to cereals in Morocco, Algeria, and Tunisia is given as 3,600,000, 3,207,-000, and 1,650,000 hectares, respectively. In addition, these countries produce large quantities of peas, beans, olives, grapes, citrus, figs, and dates. One is impressed that our present information on the agriculture of Africa is far from being complete, and that much more surveying needs to be done if we are to know what the real possibilities are.

Industrial Chemistry. Third Edition. By WILLIAM THORNTON READ. John Wiley and Sons, Inc., New York, 1943. Pp. 631, figs. 92. Price, $5.

Raw materials, processes, chemical-engineering operations, and the final products are discussed in this comprehensive volume dealing with water, fuels, lubricants, sulfur, fixed nitrogen, silicates, alkalies, fertilizers, acids, metallurgy, petroleum, oils and fats, carbohydrates, proteins, rubber, coal, dyestuffs, plastics,

explosives, and protective coatings. The beginning chapters deal with sources of information, chemical organization, the activities of chemists and chemical engineers, and the relation of chemistry to industry. Almost every chemist will find some phase of his field of endeavor covered in this volume. The material is concisely and attractively presented.

Proceedings of the Eighteenth Annual Meeting of the National Joint Committee on Fertilizer Application. H. R. Smalley, General Secretary, The National Fertilizer Association, Washington, D. C., 1943. Pp. 151.

A multigraphed report of the meetings of the National Joint Committee on Fertilizer Application that were held at St. Louis, Missouri, November 9, 1942. The report includes the data presented by the several cooperators, most of which were developed in an effort to determine what constitutes the best fertilizer placement. Of particular interest is an article dealing with the salt index of fertilizers that was presented by workers in the Bureau of Plant Industry, U. S. Department of Agriculture.

Soil Phenomena as Evidence of Climatic Changes. By KIRK BRYAN AND CLAUDE C. ALBRITTON, JR. American Journal of Science, Vol. 241, 1943. Pp. 469–490.

An article of special interest by reason of its presentation of a "promising though as yet little used method for investigating climatic changes of recent geologic time." The paper is concerned chiefly with soil phenomena in Trans-Pecos Texas, a semi-arid region along the border "between climatic regions having iron and lime subsoils."

THE EDITORS.
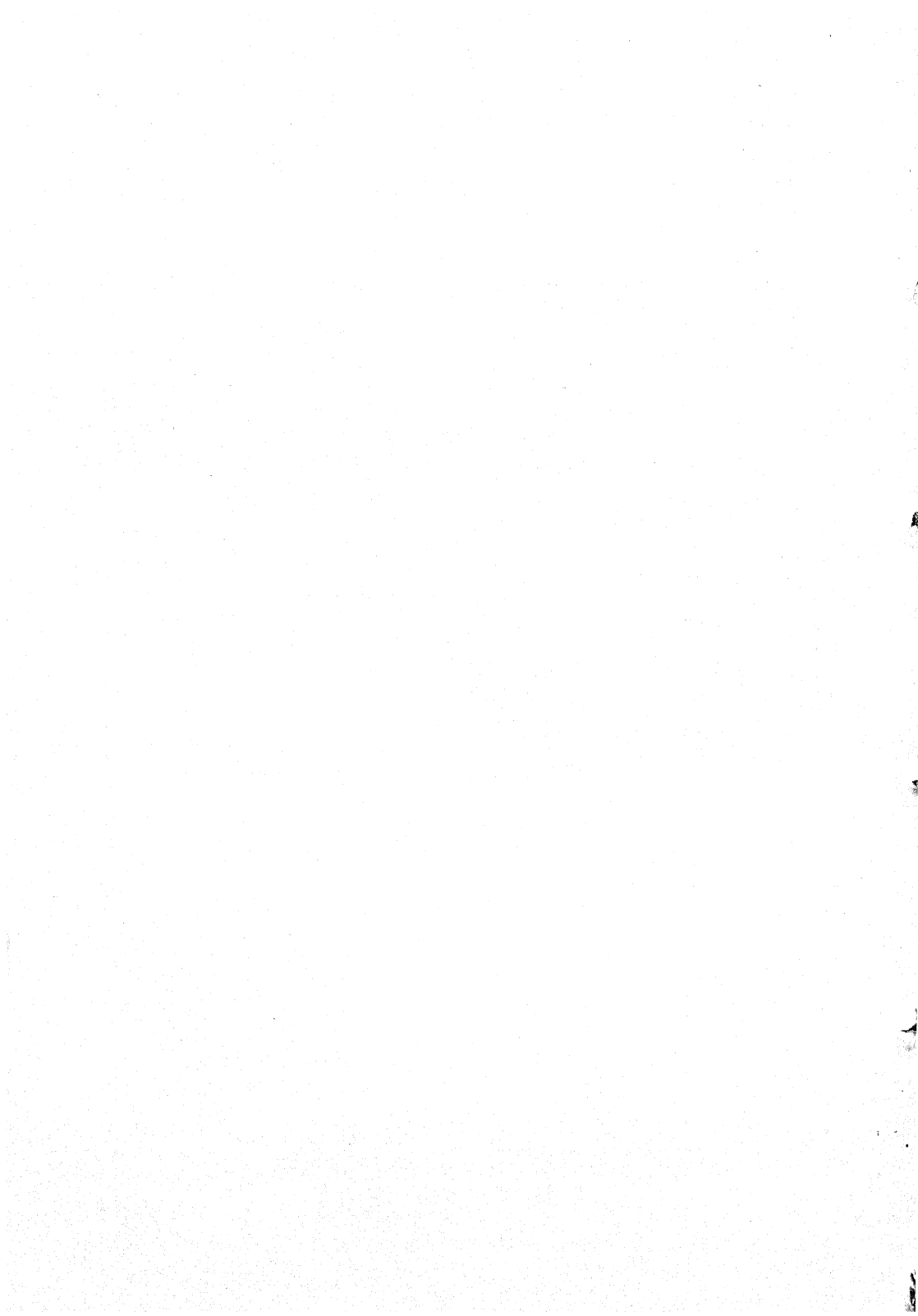
RAPID MICROCHEMICAL SOIL TESTS[1]

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The widespread interest during the last decade in the application of rapid microchemical tests to the determination of readily soluble plant nutrient elements in soils for purposes of estimating the fertilizer needs of soils and for diagnosing crop failures has led to the development of several soil testing schemes of this nature. The microchemical soil tests, often referred to as "quick soil tests" and "soil testing kits," now in use in this country, have been examined by Anderson and Noble (1) and reviewed more recently by Morgan (21). The soil testing schemes reported in the literature differ primarily in the extraction technique, but the proposed microchemical tests are essentially the same.

The chief advantage of these microchemical soil tests over the classical and the more conventional methods lies only in their simplicity and the rapidity with which the individual tests can be carried out. It is this feature that makes them especially well suited for practical routine soil testing, for which purpose the use of the more tedious classical chemical methods would be impractical and the cost prohibitive. In this respect, even the more expedient micromethods, such as those described by Peech (24) and Reitemeier (25), obviously have restricted applicability.

Unlike the classical methods of soil analysis involving a number of different extractions and laborious analytical separations, the rapid microchemical soil tests are carried out directly on separate aliquots of a single soil extract by means of colorimetric and turbidimetric methods without prior treatments or separations. Unfortunately, only very few of the inorganic or organic reagents employed in analytical chemistry possess a sufficient degree of specificity to permit their direct use in the presence of diverse ions. Although it has been generally recognized that at least some of the microchemical soil tests, as they are commonly employed, are subject to certain analytical errors, little attention has been given to possible errors caused by interfering ions. Indeed, the chemical accuracy and the reliability of the results of such tests seldom have been questioned.

In recent discussions of the limitations of the rapid chemical soil and plant tissue tests, there has been a tendency to minimize the analytical errors inherent in the tests themselves. It is often implied that there are so many biological and environmental factors involved in the interpretation and in the practical use of the soil tests that relatively little reliance can be placed on the results of the tests, however accurate they may be, and hence, no great accuracy is required. Obviously, such a fallacious viewpoint may be carried to the point where the results become so questionable as no longer to justify the expenditure of time in making the tests. It is undeniable, of course, that the results of chemical soil tests

provide only a part of the information necessary for an intelligent fertilizer recommendation. Nevertheless, chemical soil tests, when properly correlated with crop responses to fertilizers on different soils, can furnish valuable and otherwise unobtainable information that can serve a very useful purpose in fertilizer recommendations and in general diagnostic work, provided, of course, that the tests can be relied upon to give consistently accurate and reliable analytical results.

The experience in this laboratory over a period of several years with some of the rapid chemical soil tests in current use has led to the belief that the poor correlations frequently observed between the results of chemical soil tests and the crop responses to fertilizers may definitely be attributed in many instances to the analytical errors inherent in the soil tests. Accordingly, a critical examination of the rapid chemical soil tests was undertaken by the authors in an attempt to improve the accuracy of these tests without sacrificing their simplicity or speed. Among some of the inherent difficulties met with, interferences by diverse ions in many tests were found to cause sufficiently serious analytical errors to invalidate the results. These interferences were largely eliminated by the adoption and development of more specific reagents and by the use of competitive complex formers. The accuracy as well as the working range of the tests, particularly those utilizing color-läke formation, was also considerably increased by the introduction of protective colloids. The slow deterioration, with time, of some of the reagents employed as well as the significant influence of temperature variation in certain tests necessitated the adoption of natural standards in color and turbidity comparisons. Although some of the difficulties encountered in the course of the work could not be totally surmounted, it is believed that the most serious analytical errors have been alleviated and that the rapid microchemical soil tests herein described for calcium, magnesium, potassium, manganese, iron, aluminum, phosphorus, nitrate nitrogen, and ammonia nitrogen are sufficiently accurate, sensitive, and rapid for practical routine soil testing. It is the purpose of the present paper to present a description of these improved microchemical soil tests together with a discussion of interferences and other inherent analytical errors encountered in the course of the work.

DESCRIPTION OF THE MICROCHEMICAL SOIL TESTS

Apparatus

Illuminator. The illuminator that has been successfully employed in this laboratory is a simple adaptation of the "titration lamp" consisting of a 15-watt daylight fluorescent lamp in a parabolic reflector. The "titration lamp" can be obtained from several laboratory supply houses. It provides an excellent source of well-diffused indirect white light and, when used in the horizontal position with a glass window over the opening, also serves as a support for the block comparator. The glass window (16 by 12 inches) consists of two glass plates between which is placed the black line turbidity chart. This chart (12 by 4 inches) is made by drawing with India ink three sets of uniform black lines about 1, 2, and 3 mm. in width, respectively, across a sheet of cellulose acetate. The tracing cellulose acetate sheeting, etched on one side to facilitate drawing, is very satisfactory for this purpose. When

making turbidity comparisons, the vials are viewed from the top against the black lines on the chart.

Block comparator. The wooden block comparator, 13 by $1\frac{1}{2}$ by 1 inch, contains 12 vertical holes, $\frac{3}{4}$ inch in diameter and 1 inch apart on centers, bored through the entire depth of the block. The comparator is provided with a removable glass bottom plate, which is held in place by two rubber bands, to facilitate occasional cleaning. This block comparator serves to hold the vials in both turbidity and color comparison. The vials containing the series of standards are inserted in every other hole leaving the adjacent holes for the unknown test solution. The exact position of the test solution relative to the two adjacent standards can be thus readily estimated by viewing the vials from the top.

A similar block comparator, 13 by $1\frac{1}{2}$ by 1 inch, made to accommodate the smaller vials, is provided with 12 holes, $\frac{5}{8}$ inch in diameter, and $\frac{7}{8}$ inch apart on centers.

Vial racks. For large vials, a rack with the following specifications is suggested: 13 by 5 by $1\frac{3}{4}$ inches; 48 holes, $\frac{3}{4}$ inch in diameter, $\frac{3}{4}$ inch in depth, and 1 inch apart on centers. For small vials the specifications are: 13 by 5 by $1\frac{1}{4}$ inches; 48 holes, $\frac{5}{8}$ inch in diameter, $\frac{3}{4}$ inch in depth, and $\frac{7}{8}$ inch apart on centers.

Water bath. A water bath with a removable rack, made of sheet copper, to accommodate 48 vials (65 by 19 mm.), can be used to advantage when temperature control becomes necessary in making the calcium and potassium tests.

Reciprocating shaker. The use of some form of mechanical shaking device during extraction not only is more expedient than hand shaking but also assures greater reproducibility of results. The common box-type reciprocating shaker equipped with drawers and compartments to fit the shaking bottles is quite convenient.

Vials. Ordinary "specimen" shell vials of clear glass with flat bottoms are selected for uniformity. Two different sizes have been found satisfactory: large vials, 65 by 19 mm.; small vials, 50 by 16 mm.

Pipettes. Measuring pipettes: 5- and 10-ml. pipettes subdivided to $\frac{1}{10}$ ml. Serological pipettes: 1-ml. pipette subdivided to $\frac{1}{100}$ ml., and $\frac{1}{10}$-ml. pipette subdivided to $\frac{1}{100}$-ml. With the exception of the 10-ml. measuring pipette, these pipettes are used for measuring out the aliquots of soil extracts. It is expedient, therefore, to have a sufficient number on hand to provide an individual pipette for each soil extract.

Dropping bottles. 60-ml. bottles of amber glass provided with a pipette and a rubber bulb.

Extraction

Extracting solution. Dissolve 100 gm. of $NaC_2H_3O_2 \cdot 3H_2O$ in about 500 ml. of water, add 30 ml. of 99.5 per cent acetic acid, and dilute with water to 1 liter. It is expedient to prepare 19 liters of this solution at a time in a 5-gallon Pyrex bottle previously marked to contain 19 liters.

Procedure. To 10 gm. of soil (air-dried and screened through a 1-mm. sieve) in a shaking bottle, add $\frac{1}{4}$ measuring teaspoonful ($\frac{1}{4}$ gm.) of activated carbon (Darco G 60) and 50 ml. of the extracting solution. When extracting organic soils, the amount of carbon added should be increased to $\frac{1}{2}$ teaspoonful. Shake for 30 minutes, and filter through 11-cm. paper (American S & S No. 597 or Whatman No. 32) into a 50-ml. Erlenmeyer flask.

The p.p.m. of any constituent in the extract $\times$ 10 = pounds per acre (2,000,000 pounds of soil).

Preparation of standards

In preparing the necessary standards shown in table 1, the proper amounts of the respective standard solutions are measured out into the vials, diluted with the extracting solution to the volume of the aliquot called for in the test, and subjected simultaneously to the same procedure. If the amount of constituent found is greater than that of the highest standard, the determination is repeated on a smaller aliquot after dilution to appro-

priate volume with the extracting solution. The result, of course, should be multiplied by the dilution factor.

TABLE 1

Preparation of standards for rapid microchemical soil tests

CONSTITUENT	STANDARD SOLUTION	EXTRACTING SOLUTION	AMOUNT PER ACRE	CONSTITUENT	STANDARD SOLUTION	EXTRACTING SOLUTION	AMOUNT PER ACRE
	ml.	*ml.*	*lbs.*		*ml.*	*ml.*	*lbs.*
Ca	0	0.50	0	Fe	0	1.00	0
	0.10	0.40	400		0.10	0.90	10
	0.20	0.30	800		0.20	0.80	20
	0.30	0.20	1200		0.40	0.60	40
	0.40	0.10	1600		0.60	0.40	60
	0.50	0	2000		0.80	0.20	80
Mg	0	3.00	0	Al	0	1.00	0
	0.30	2.70	25		0.10	0.90	25
	0.60	2.40	50		0.20	0.80	50
	1.20	1.80	100		0.40	0.60	100
	1.80	1.20	150		0.60	0.40	150
	2.40	0.60	200		1.00	0	250
	3.00	0	250				
K	0	2.00	0	P	0	4.00	0
	0.25	1.75	50		0.20	3.80	5
	0.50	1.50	100		0.40	3.60	10
	0.75	1.25	150		0.80	3.20	20
	1.00	1.00	200		1.60	2.40	40
	1.25	0.75	250		2.40	1.60	60
	1.50	0.50	300		3.20	0.80	80
Mn	0	2.00	0	NH₃-N	0	1.00	0
	0.25	1.75	25		0.125	0.87	25
	0.50	1.50	50		0.25	0.75	50
	1.00	1.00	100		0.50	0.50	100
	1.50	0.50	150		0.75	0.25	150
	2.00	0	200		1.00	0	200
				NO₃-N	0	0.50	0
					0.10	0.40	20
					0.20	0.30	40
					0.30	0.20	60
					0.40	0.10	80
					0.50	0	100

Determination of calcium

Reagents.[2] 1. Standard calcium solution (200 p.p.m. Ca): Dissolve 0.879 gm. of $Ca(C_2H_3O_2)_2 \cdot H_2O$ in 1 liter of the extracting solution. Preserve this solution as well as other standard solutions by the addition of 1 ml. of chloroform per liter of solution. It is

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[2] All chemicals unless otherwise specified are c. p. or of reagent grade quality.

advisable to standardize this solution as well as the magnesium acetate solution listed under the magnesium determination as a check on the water of crystallization of the respective salts.

2. Soap solution: In a 2-liter Erlenmeyer flask, dissolve 0.60 gm. of recrystallized stearic acid (Eastman reagent No. 402 or similar quality) and 7.5 ml. of oleic acid (U. S. P.) in 320 ml. of 95 per cent ethyl alcohol by heating on a hot plate. Add 16 gm. of ammonium carbonate dissolved in 80 ml. of hot water and boil for about 10 minutes. Cool, add 360 ml. of 95 per cent ethyl alcohol, 40 ml. of water, and 1.6 ml. of concentrated ammonium hydroxide. Filter after 24 hours and store in a Pyrex glass-stoppered bottle.

3. Ammoniacal citrate solution: Dissolve 1.5 gm. of sodium citrate ($2Na_3C_6H_5O_7 \cdot 11H_2O$) in water, add 14 ml. of concentrated ammonium hydroxide (sp. gr. 0.90), and dilute to 1 liter with water.

Procedure. To 0.5 ml. of the extract in a large vial (65 by 19 mm.), add 2 ml. of the ammoniacal citrate solution, mix, and then add 1.5 ml. of the soap solution down the side of the vial so as to form a layer on top of the solution. Mix rapidly and uniformly after the addition of the soap solution to all of the vials. When a large number of tests are run concurrently as is usually the case, two vials are shaken at the same time. After 30 minutes, compare with a series of standards as given in table 1 made up at the same time and in the same manner. The test should not be attempted at temperatures above 28°C.

Determination of magnesium—titan yellow procedure

Reagents. 1. Standard magnesium solution (25 p.p.m. Mg): Dissolve 0.2205 gm. of $Mg(C_2H_3O_2)_2 \cdot 4H_2O$ in 1 liter of the extracting solution.

2. Titan yellow, 0.10 per cent. Dissolve 0.10 gm. of titan yellow (Dr. Grubler & Co., or similar quality) in 100 ml. of distilled water and filter if necessary. When stored in an amber glass dropping bottle, this reagent will keep for 2 months. Apparently, other related dyes are also classified and listed as titan yellow by some chemical supply houses. Several domestic preparations have been tried and found considerably less sensitive than the German product.

3. Hydroxylamine hydrochloride, 5 per cent: Dissolve 25 gm. of hydroxylamine hydrochloride in water and dilute to 500 ml.

4. Sodium hydroxide, 2.5 N: Dissolve 100 gm. of sodium hydroxide in water and dilute to 1 liter. Store in a Pyrex glass bottle.

5. Starch solution, 2 per cent: To 2 gm. of c. p. soluble starch add a few drops of water and make a paste by stirring with a glass rod. Add slowly while stirring, 100 ml. of boiling distilled water. Filter the solution while hot through Whatman No. 31 filter paper. A satisfactory grade of soluble starch should all go immediately into solution, giving virtually a clear solution. This reagent should be freshly prepared daily as needed.

6. Compensating solution: To 4.4 gm. of $Ca(C_2H_3O_2)_2 \cdot H_2O$ and 0.37 gm. of $Al_2(SO_4)_3 \cdot 18H_2O$ in a 1-liter volumetric flask, add 10 ml. of concentrated HCl and about 500 ml. of water; mix to dissolve the salts, and dilute to volume with water.

7. Starch reagent: Prepare sufficient quantity of this reagent daily as needed by mixing equal volumes of 2 per cent starch solution (reagent 5) and compensating solution (reagent 6).

Procedure. To 3 ml. of the extract in a large vial (65 by 19 mm.), add two drops of hydroxylamine hydrochloride solution and 1 ml. of the starch reagent (No. 7); mix, add three drops of titan yellow solution, and mix again; then add 1 ml. of sodium hydroxide solution and mix thoroughly. Compare with a series of standards, as listed in table 1, prepared simultaneously in the same manner.

Determination of magnesium—p-nitrobenzeneazoresorcinol procedure

Reagents. 1. *p*-nitrobenzeneazoresorcinol reagent, 0.05 per cent: Dissolve 0.05 gm. of *p*-nitrobenzeneazoresorcinol in 1 ml. of 0.25 N NaOH, and dilute to 100 ml. with water.

2. Mannite solution, 15 per cent: Dissolve 75 gm. of mannite in water and dilute to 500 ml. Preserve by adding 1 ml. of chloroform.

3. Starch solution: 1 per cent solution of soluble starch in water.

4. Sodium hydroxide, 2.5 N.

5. Standard magnesium solution: listed under the titan yellow procedure.

Procedure. To 3 ml. of the extract in a large vial (65 by 19 mm.), add 1 ml. of mannite solution, 1 ml. of starch solution, and 1 drop of *p*-nitrobenzeneazoresorcinol reagent. Mix well; then add 1 ml. of sodium hydroxide solution and mix well again. Compare with a series of standards prepared simultaneously in the same manner.

Determination of potassium

Reagents. 1. Standard potassium solution (40 p.p.m. K): Dissolve 0.0763 gm. of KCl in 1 liter of the extracting solution.

2. Sodium cobaltinitrite reagent: In a 250-ml. volumetric flask, dissolve 6.25 gm. of $Co(NO_3)_2 \cdot 6H_2O$ and 75 gm. of $NaNO_2$ in about 175 ml. of water; add 5 ml. of 99.5 per cent acetic acid and mix very gently at first to prevent loss by foaming; cover with a beaker and allow to stand overnight to permit the escape of nitric oxide. Dilute to 250 ml., mix well, and filter. When stored in a refrigerator in a glass-stoppered Pyrex bottle, this reagent will keep for at least 1 month.

3. Isopropyl alcohol, c. p.

4. Formaldehyde, c. p. 37 per cent.

Procedure. Transfer a 2-ml. aliquot of the extract into a large vial (65 by 19 mm.). Add six drops of formaldehyde solution, mix, and allow to stand for about 5 minutes; then add 1 ml. of the sodium cobaltinitrite reagent; mix well again, then add 2 ml. of isopropyl alcohol down the side of the vial so as to form a layer of alcohol on top of the solution. It is imperative that the alcohol be added without mixing the solutions at this stage. After the last addition of alcohol to the extracts as well as to the standard solutions, mix the two layers uniformly and rapidly by swirling the vial for about 30 seconds. After about 25 minutes, compare the turbidities with a series of standards carried simultaneously through the procedure. The best results are obtained at 25° ± 4° C.; temperatures above 29°C. should be avoided.

Determination of manganese

Reagents. 1. Standard manganese solution (20 p.p.m. Mn): To 0.0288 gm. of $KMnO_4$ in a 125-ml. Erlenmeyer flask, add 10 ml. of water and six drops of concentrated sulfuric acid. Heat to boiling and add sufficient sodium bisulfite (avoid large excess) to discharge the color. Evaporate down until fumes of H_2SO_4 appear, cool, and dissolve the residue in 500 ml. of the extracting solution.

2. Sulfuric acid conc., sp. gr. 1.84.

3. Sodium bismuthate, c. p. powder.

Procedure. Transfer a 2-ml. aliquot of the extract into a large vial (65 by 19 mm.), add 0.2 ml. of concentrated sulfuric acid, and mix. After cooling to room temperature, add a pinch (about 0.1 gm.) of sodium bismuthate; shake for about 30 seconds, and allow the excess bismuthate to settle out. As soon as the excess of bismuthate has settled out, which takes about 30 minutes, compare with a series of standards by viewing through the sides of the vials against a white background.

Determination of iron

Reagents. 1. Standard iron solution (10 p.p.m. Fe): Dissolve 0.0351 gm. of $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ in about 300 ml. of extracting solution, add 2 ml. of 5 per cent hydroxylamine hydrochloride solution, and dilute to 500 ml. with the extracting solution.

2. Hydroxylamine hydrochloride: 5 per cent solution in water, already listed under the magnesium test.

3. *o*-phenanthroline: 0.5 per cent solution in 50 per cent ethyl alcohol.

Procedure. To 1 ml. of the extract in a small vial (50 by 16 mm.), add one drop of hydroxylamine hydrochloride solution and mix; then add two drops of *o*-phenanthroline solution and mix again. After 10 minutes compare with a series of standards. When stoppered and not exposed to light, the standards will keep for several months.

Determination of aluminum

Reagents. 1. Standard aluminum solution (25 p.p.m. Al): Dissolve 0.1544 gm. of $Al_2(SO_4)_3 \cdot 18H_2O$ in 500 ml. of the extracting solution.

2. Aluminon, 0.2 per cent: Dissolve 0.20 gm. of aluminon (ammonium salt of aurintricarboxylic acid) in 100 ml. of water. Store in an amber glass dropping bottle; prepare a fresh supply of this reagent every 6 months.

3. Starch solution: 1 per cent solution of soluble starch in water. Usually a sufficient amount of 2 per cent starch solution is prepared when making the magnesium determinations to serve the purpose here after dilution with equal volume of water.

4. Hydroxylamine hydrochloride, 5 per cent: listed under the magnesium test.

Procedure. To a 1-ml. aliquot of the extract in a large vial (65 by 19 mm.), add two drops of the hydroxylamine hydrochloride solution and mix; add 3 ml. of water and 1 ml. of the starch solution, and mix again; then add two drops of the aluminon reagent and mix thoroughly. Allow to stand for at least 30 minutes before comparing with a series of standards subjected simultaneously to the same procedure.

Determination of phosphorus

Reagents. 1. Standard phosphate solution (10 p.p.m. P): Dissolve 0.0439 gm. of KH_2PO_4 in 1 liter of the extracting solution.

2. Molybdate reagent: In a 1-liter volumetric flask, dissolve 15 gm. of ammonium molybdate $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in about 300 ml. of distilled water. Add slowly while shaking, 500 ml. of concentrated hydrochloric acid, sp. gr. 1.18. Cool to room temperature, dilute with water to 1 liter, and mix. Store in a brown glass-stoppered bottle. Prepare a fresh supply of this reagent every 3 months.

3. Stannous oxalate solution: Dissolve 0.5 gm. of stannous oxalate in 100 ml. of dilute HCl (1 part conc. HCl + 9 parts water). Filter through a Whatman No. 40 filter paper into a separatory funnel. Cover the solution with about 1.5 cm. layer of paraffin oil. This reagent will keep for about 1 month.

Procedure. To 4 ml. of the extract in a large vial (65 by 19 mm.), add rapidly from a burette, against the side of the vial, 1 ml. of the molybdate reagent and mix well immediately. After the addition of the molybdate to all of the extracts and the standards, add three drops of stannous oxalate solution, and mix well immediately. Compare with a series of standards prepared simultaneously in the same manner.

Determination of ammonia

Reagents. 1. Standard ammonia nitrogen solution (20 p.p.m. N): Dissolve 0.0471 gm. of $(NH_4)_2SO_4$ in 500 ml. of the extracting solution.

2. Nessler reagent: In a 500-ml. volumetric flask, dissolve 45.5 gm. of mercuric iodide and 35.0 gm. of potassium iodide in as little water as is needed. Add 112 gm. of KOH, mix well, cool, and dilute to 500 ml. Allow any precipitate to settle out for a few days before using. Store in a brown glass bottle.

3. Sodium hydroxide-tartrate solution: Dissolve 40 gm. of sodium tartrate $(Na_2C_4H_4O_6 \cdot 2H_2O)$ in about 300 ml. of water; add 13 gm. of sodium hydroxide, and after sodium hydroxide has dissolved, dilute to 500 ml. with water.

4. Gum acacia solution, 5 per cent: Dissolve 10 gm. of powdered gum acacia in 195 ml. of water, and then add 5 ml. of the Nessler reagent. Gum acacia may be dissolved most readily by adding just sufficient water to make a paste and then adding the balance of the

water while stirring. Allow to settle for a few days before using, and store in a brown glass bottle. Prepare a fresh supply of this solution every 2 months.

Procedure. Transfer a 1-ml. aliquot of the extract into a large vial (65 by 19 mm.), add 2 ml. of water, and mix; then add 1 ml. of the sodium hydroxide-tartrate solution down the side of the vial to all of the extracts as well as to the standard solutions. Mix well and immediately add four drops of gum acacia solution and two drops of Nessler reagent, mixing well after last addition. After 15 minutes compare with a series of standards prepared in the same manner and at the same time. The 200-pound-per-acre standard shown in table 1 is seldom required.

Determination of nitrate

Reagents. 1. Standard nitrate solution (10 p.p.m. N): Dissolve 0.0361 gm. of KNO_3 in 500 ml. of the extracting solution.

2. Brucine, 4 per cent: Dissolve 4 gm. of brucine in 100 ml. of chloroform and store in a closely stoppered amber glass dropping bottle.

3. Sulfuric acid, conc., sp. gr. 1.84.

Procedure. To 0.5 ml. of the extract in a small vial (50 by 16 mm.), add three drops of brucine solution and 1 ml. of concentrated sulfuric acid. Mix well, and after 15 minutes compare with a series of standards carried simultaneously through the procedure.

Determination of soil reaction (pH)

To 20 gm. of soil in a 50-ml. beaker, add 20 ml. of distilled water, and stir the suspension several times at regular intervals for about an hour. Measure the pH of the soil suspension with the glass electrode, stirring well just prior to immersing the electrode in the suspension.

Supplementary soil tests

Other rapid soil tests made in this laboratory include the determination of soil organic matter by the chromic acid titration method (33) and the conductometric determination of total soluble salts, the latter being primarily used for testing the salinity of greenhouse soils. The conductivity measurements are made on 1:2 soil-water suspensions at 25°C., and the results are expressed in terms of specific conductance as reciprocal ohms ($K \times 10^5$).

RESULTS AND DISCUSSION

Extraction

Nearly all of the rapid microchemical soil testing schemes in current use employ only a single extracting solution for the determination of the different constituents, primarily for the sake of expediency. Many restrictions necessarily are imposed on the choice of the soil extracting solution best suited to the determination of all the constituents in question. The extracting solution must remove only the nutrients that are readily available to the plant, and the results of the test must necessarily correlate with known crop responses to fertilizers. In order to conform with the accepted chemical procedures, a satisfactory extracting solution should effect complete removal of the exchangeable bases. It should yield clear extracts free of organic matter and, therefore, must be acidic in character. The extracting solution should not contain ions that will interfere with the proper execution of any of the individual tests. The concentrations of all the ions under examination must be within limits of acceptable sensitivity of the respective tests. Obviously, such an ideal soil extracting solution is wholly fictitious.

After considerable preliminary work, Morgan's (21) universal soil extracting solution, which is now in rather extensive use, was chosen. This is a 10 per cent solution of sodium acetate ($0.73N$) in 3 per cent acetic acid ($0.52N$) having a pH of 4.8. As pointed out by Morgan, it is well buffered at a pH value that is closely approximated by that of the soil solution in equilibrium with the partial CO_2 pressure normally found in the soil air, and its solvent action is relatively unchanged even upon prolonged contact with a soil containing moderate amounts of calcium carbonate. This solution has been found capable of replacing a fairly high proportion of the exchangeable bases in most soils by a single extraction using 1 part of soil to 5 parts of the extracting solution, as shown in table 7, and in this respect, is superior to the similar but weaker sodium acetate solutions employed by Hester, Blume, and Shelton (13) and Merkle (18).

One of the objectionable features of the extracting solutions of strong mineral acids, such as 0.3 N HCl employed by Truog[3] and Baver and Bruner (2), lies in the unduly large amounts of dissolved iron and aluminum which, in addition to interfering seriously with many tests, may cause partial reprecipitation of the dissolved phosphorus during the extraction process. Because of the capacity of the acetate ion to form complexes with iron and aluminum, there is little if any reprecipitation of the dissolved phosphorus from sodium acetate solutions at pH 4.8 even in the presence of relatively large amounts of iron and aluminum. In fact, standard synthetic soil solutions, which were made up in Morgan's extracting solution to contain ferric, aluminum, and phosphate ions in greater concentrations than are normally found in soil extracts, have been kept for 6 months without any evidence of precipitation. Despite the fact that the pH of Morgan's extracting solution is virtually unchanged even in the extraction of soils containing a moderate excess of $CaCO_3$, very little iron or aluminum is liberated by this solution from slightly acid or neutral soils, whereas large amounts of iron and especially of aluminum are usually extracted from the more acid soils, as shown in table 7. It is believed that the greater part of the iron and aluminum extractable by this solution represents water-soluble and exchangeable forms and that the presence of these two constituents is, therefore, indicative of unfavorable soil conditions such as extreme acidity and poor aeration. On the other hand, little, if any, practical or ecological significance can be attached to the presence of iron or aluminum in soil extracts of stronger mineral acids.

It will be noted from table 7 that the amount of manganese extracted by Morgan's solution from most soils considerably exceeds the exchangeable manganese content as determined by the ammonium acetate method (24). The nonexchangeable manganese soluble in Morgan's extracting solution probably represents some forms of manganic oxides.

At first thought it might appear that the large amounts of nonexchangeable manganese liberated by Morgan's solution would tend to mask any real significance of the exchangeable manganese. This, however, is not necessarily true in the case of this constituent; the exchangeable manganese, being largely

[3] Truog, E.   Abbreviated directions for Hellige-Truog combination soil tester.   Mimeographed.

controlled by soil reaction and the oxidation-reduction status of the soil, is subject to considerable fluctuation and is not always a good criterion of the available manganese supply of soils. Sherman, McHargue, and Hodgkiss (27) consider the "easily reducible" manganese oxides as an important source of manganese for plants when the exchangeable manganese content is low.

The amounts of phosphorus extracted from soils by Morgan's solution are considerably lower than the amounts removed by other extracting solutions employing stronger mineral acids, such as Truog's $0.002N$ H_2SO_4 (30), as shown in table 7. Inasmuch as all chemical methods for the determination of readily soluble phosphorus in soils are highly empirical and must be thoroughly correlated against crop responses to phosphate fertilization, there is little choice among them, as recently noted by Weeks and Karraker (34). In comparing several chemical methods for determining phosphate needs of western irrigated soils, however, Dahlberg and Brown (6) found that the sodium acetate-acetic acid solution at pH 5 gave the best results.

Although the use of any single extracting solution for the determination of all the constituents in question is inevitably a more empirical approach than the employment of several different extracting solutions for the same purpose and is not likely to give results that are in very good agreement with those obtained by the more thoroughly established soil analytical methods now in use, it should be obvious from the foregoing considerations that the sodium acetate-acetic acid solution proposed by Morgan has certain desirable features not possessed either by solutions of strong acids or by solutions of neutral salts. In tentatively adopting this extracting solution, however, it was realized that extraction methods could not be satisfactorily compared until accurate microchemical tests for the various constituents were available.

Most of the rapid soil testing schemes employ the equilibrium extraction technique in the preparation of soil extracts, although Morgan (21) favors the percolation procedure. The proportion of soil to the extracting solution commonly employed is 1:2.5. Merkle (18) uses a 1:5 ratio. Such narrow ratios of soil to extracting solution are employed, despite incomplete extraction of the exchangeable bases, in order to have the concentrations of all the constituents under examination within the limits of sensitivity of the microchemical tests. For this reason, percolation procedures requiring rather large volumes of extracting solution are not suitable. Attempts to leach the soil samples with smaller volumes of extracting solution as recommended by Morgan (21) were made by the authors, but the results obtained by such procedures were usually erratic. The equilibrium extraction technique using 1:5 soil:extracting solution ratio was, therefore, adopted. The refinements in the microchemical tests, as already noted, made possible the adoption of this ratio of soil to extracting solution without a sacrifice in the sensitivity of any of the tests. Experiments with six widely different soils which had been air-dried and stored for over a year showed that the time of extraction (shaking) may be reduced to 15 minutes without affecting the results. Extraction for 30 minutes, however, as suggested in the procedure, is likely to give more reproducible results with air-dry soils

that are difficult to wet; for the same reason, extraction for very short periods, 2 to 5 minutes, as commonly employed, is not recommended.

Removal of soluble organic matter

Despite its acidity, Morgan's soil extracting solution was found to bring sufficient organic matter into solution to interfere seriously with some of the tests. The soluble organic matter in the extracts imparted interfering off-color tints in the phosphorus, magnesium, and, to a lesser extent, other colorimetric tests and thus made the matching of colors very difficult or impossible. Its reducing action in the oxidation of manganese by bismuthate rendered this test worthless even in the presence of relatively small amounts of soluble organic matter. Another type of interference by organic matter, perhaps less serious than those already mentioned, was observed in the turbidimetric tests for calcium and potassium. Inasmuch as particle size, which in turn is affected by the rate of precipitation, is one of the important factors in the development of turbidity, it is quite probable that the interferences in the turbidimetric tests were due

TABLE 2

Recoveries of added constituents from a standard synthetic soil solution,* before and after treatment with activated carbon, as obtained by the proposed tests

Pounds per acre (2,000,000 pounds of soil)

CONSTITUENT	Ca	Mg	K	Mn	Fe	Al	P	NO₃-N	NH₄-N
Theoretical amount present	1470	170	220	90	62	155	68	48	100
Amount found before treatment with carbon	1500	125	225	85	60	150	65	50	110
Amount found after treatment with carbon	1500	125	225	85	30	125	60	50	110

* Prepared from calcium and magnesium acetates; ammonium, manganous, ferric, and aluminum sulfates; potassium as the primary phosphate and nitrate.

either to the peptizing or to the inhibiting action of organic matter in the precipitation process. The possibility of removing the dissolved organic matter by means of activated carbon was then investigated. Of the several carbons tried, activated carbon (Darco G60, obtained from Darco Corporation) was found sufficiently free of impurities and very effective in decolorizing the extracts.

In order to determine whether any of the ions under examination were adsorbed by this carbon, 50 ml. of standard synthetic soil solution, made up in the extracting solution, was shaken for 30 minutes with 0.25 gm. of carbon, filtered, and examined by the tests herein described. The results are listed in table 2. With the exception of ferric and aluminum ions, which were partly adsorbed, very satisfactory recoveries of other ions were obtained. Subsequent tests have shown that the ferrous ion is not adsorbed and that there is a slight but negligible adsorption of phosphate ion presumably as complex ferric or aluminum phosphate only in the presence of relatively large amounts of ferric iron and aluminum, as shown in table 2. Inasmuch as ferric and aluminum ions form

fairly stable complexes in acetate solutions, it may be concluded that within limits of the experimental error of the tests, this activated carbon does not adsorb simple inorganic ions in true solution, at least not in the presence of the high concentration of sodium acetate in the extracting solution employed. Although most of the iron extracted by Morgan's solution from well-aerated soils is in the ferric state, the amounts are relatively small; much larger amounts of soluble iron, however, are found in poorly drained soils, particularly under conditions conducive to reduction, and in such soils the greater part of the soluble iron is usually in the ferrous state. Thus, the two constituents that are likely to be adsorbed by carbon, especially when present in large amounts, are ferric iron and aluminum. It is questionable, however, whether this source of error should alter the interpretation of the relative practical significance of the results of the iron and aluminum tests. In the more accurate studies on iron and aluminum in soil extracts, it is recommended that the organic matter be removed by other means. Because of the many different types of activated carbons now available on the market for specific purposes, it is advisable to check every new source of carbon for possible adsorption or release of ions under examination. Different lots of Darco G60 activated carbon examined thus far have been found quite uniform and satisfactory.

Determination of calcium

In all of the rapid microchemical soil tests in current use, calcium is estimated turbidimetrically as the oxalate. The accuracy of this method has been questioned recently by Melsted (17). The authors' experience with the oxalate method for the rapid estimation of calcium also indicates that this procedure lacks the necessary precision and is subject to large experimental errors which in some cases may be as high as 100 per cent. The pH of the solution, the presence of soluble organic matter and diverse ions, and the manner of adding and mixing the reagents were found to be the important factors in the development of the final turbidity. Likewise, the presence of magnesium has been reported (15) to influence the crystal form of calcium oxalate and, consequently, the turbidity of the suspension. Hence, the final turbidity is a function not only of the amount of precipitate but also of the particle size and the crystal form. Furthermore, the precipitate settles entirely too rapidly to permit accurate comparison of turbidity with a series of standards. Unsuccessful attempts were made to induce more rapid precipitation at pH 8 in order to obtain a more finely divided precipitate, the procedure employed by Merkle (18), both in the absence and in the presence of stabilizers such as glycerol as suggested by Baver and Bruner (2).

Several soap reagents that have been proposed for the turbidimetric determination of calcium as the oleate (29), sulforicinoleate (29), or the stearate (16) were then examined, but none were found satisfactory. These methods either lacked the proper sensitivity over the desired range of calcium concentration or the reagents were too unstable in the presence of the high concentration of sodium acetate in the extracting solution. Also, in most turbidimetric soap methods reported in the literature, calcium must be first separated from magnesium and other interfering ions.

Ammonium stearate prepared from a technical grade of stearic acid according to the directions given by Lyman (16) gave fairly satisfactory results only when the ratio of the volume of the reagent to the test solution was reduced from 2.5, as recommended by Lyman, to 1, but even under this condition the soap flocculated and formed a gel immediately after mixing with the test solution at temperatures below 23°C. The reagent prepared from purified stearic acid, however, proved very unstable upon dilution with the test solution, apparently because of hydrolysis of ammonium stearate and consequent separation of stearic acid to form a gel even in the presence of considerable excess of ammonium hydroxide. Increasing the amount of reagent tended to prevent the hydrolysis of ammonium stearate but at the same time caused almost immediate flocculation of calcium stearate. This led to a careful study of the composition of the reagent in an effort to determine the optimum amounts of stearic acid, oleic acid (used for stabilization), alcohol, and ammonium hydroxide necessary to give the maximum stability and the greatest sensitivity within the desired range of calcium concentration. Consequently, the soap reagent described under the calcium test was finally adopted. In addition to being considerably more stable, this reagent is

TABLE 3

Effect of diverse ions on the proposed turbidimetric soap test for calcium

CONSTITUENT PRESENT	Ca FOUND	CONSTITUENT PRESENT	Ca FOUND
lbs./A.	*lbs./A.*	*lbs./A.*	*lbs./A.*
1000 Mg....................	0	300 Al........................	0
500 Mg + 1500 Ca.............	1500	150 Al + 1500 Ca.............	1500
100 Fe^{+++}....................	0	200 Mn^{++}....................	0
100 Fe^{+++} + 1500 Ca..........	1500	100 Mn^{++} + 1500 Ca..........	1500

also less sensitive to magnesium than the ammonium stearate reagent described by Lyman. Furthermore, the authors found that in the presence of a small amount of citrate this reagent is indeed very specific for calcium, as shown in table 3, giving no soap cloud with magnesium, manganese, iron, or aluminum even when these ions are present in concentrations greater than are normally found in soil extracts.

This new turbidimetric calcium test has given very reproducible and accurate results. By using the aliquot and the size of vial as directed in the procedure, calcium may be estimated within 100 pounds in the working range from 0 to 2000 pounds per acre. Because of the rather abrupt decrease in sensitivity of the test above 28° C., the temperature of the test solutions and reagents should be kept below 28° C., preferably between 20° and 26° C. Unlike the calcium oxalate in the turbidimetric estimation of calcium, the colloidal calcium stearate remains in suspension without any settling for two or three hours, after which time slight flocculation usually occurs only at higher calcium concentrations.

Determination of magnesium

Two of the better organic reagents for direct colorimetric determination of

reagents, titan yellow has been used more extensively in the rapid microchemical soil tests. As a result of adsorption of the dye by magnesium hydroxide, both reagents form color lakes with magnesium in alkaline solution. Except in very dilute magnesium solutions the lakes precipitate immediately unless some suitable protective colloid is present. Although as a rule the color intensity of color lakes is not affected by dispersing agents, the use of a protective colloid in the formation of the magnesium lakes by these two reagents, particularly titan yellow, results in considerable intensification of the color. Hence, the use of a protective colloid in the magnesium test employing either of these two reagents serves not only to extend considerably the range of the test but also increases the sensitivity. Among the protective colloids tried, soluble starch and gum tragacanth were both found equally satisfactory, but since the latter must be purified by electrodialysis or other means, starch is, therefore, recommended. The rapid fading caused by some samples of starch as previously reported by Peech (23) in the titan yellow method has not been encountered here, the apparent discrepancy being due to the presence of oxalate formerly used for the removal of calcium prior to making the magnesium test.

The titan yellow as well as the p-nitrobenzeneazoresorcinol test for magnesium was found to be more subject to serious errors caused by interfering ions than any other test investigated. Calcium, manganese, aluminum, and, to a lesser extent, iron were found to interfere with both tests. Inasmuch as attempts to eliminate or minimize the action of interfering ions in the two cases were not equally successful, the interferences in the two tests will be discussed separately.

Titan yellow test. Although calcium, regardless of concentration, does not give a color lake with titan yellow in the absence of magnesium, calcium intensifies or decreases the color intensity of the magnesium lake, thus introducing a positive or a negative error depending on the amount of magnesium present. For example, with 50 pounds per acre of magnesium present,[4] the addition of as much as 4000 pounds of calcium was found to have no effect on the color intensity of the magnesium lake. On the other hand, with 200 pounds of magnesium and 1000 pounds of calcium present, the color of the magnesium lake was markedly intensified by calcium, the effect of 4000 pounds of calcium in this case being the same as that of 1000 pounds. But when the amount of magnesium present was in excess of 400 pounds, the effect of increasing the amount of calcium was to decrease the color intensity of the magnesium lake. In an effort to overcome the interfering action of calcium, the addition of citrate, tartrate, sucrose, mannite, and other complex formers proved to be of little or no value. Sucrose, which has been employed for this purpose by Baver and Bruner (2) and by Gillam (10),

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[4] To facilitate discussion and to avoid possible confusion of "p.p.m." of any given constituent in the original extract with the final concentration of that constituent in the test solution after the addition of all reagents, the more conventional expression "pounds per acre" (2,000,000 pounds of soil) will be retained throughout the discussion to designate the amount of constituent present in the synthetic solution as based on the full aliquot called for in the procedure. Thus, 50 pounds of Mg is equivalent to 5 p.p.m. of Mg in the 3-ml. aliquot of the original extract or to 2.86 p.p.m. in the final test solution after color development.

partly eliminated the calcium interference when the amount of magnesium present was less than 200 pounds per acre. With larger amounts of magnesium, calcium caused a negative interference in the presence of sucrose. For example, when 1 ml. of 10 per cent sucrose solution was added to the test solution containing an equivalent of 400 pounds of magnesium and 2000 pounds of calcium per acre, the actual amount of magnesium found by comparison with standards containing the same amount of sucrose was 60 per cent too low. The best way, found thus far, of preventing calcium interference with this test is to add to the standards as well as to the soil extracts a sufficient amount of calcium to produce maximum intensification of the color of the magnesium lake, and to repeat the test on a smaller aliquot of soil extract when the amount of magnesium found is over 200 pounds per acre.

The behavior of aluminum in this test was found to be similar to that of calcium. Aluminum alone gave no color lake with titan yellow but markedly intensified the color of the magnesium lake when the amount of magnesium was greater than 50 pounds per acre. With smaller amounts of magnesium, the presence of aluminum was without effect. Fortunately, it was found that maximum intensification of the magnesium color lake could be attained by the addition of aluminum equivalent to 50 pounds per acre. Upon further addition of aluminum, the color intensity remained comparatively constant except when magnesium was present in excess of 200 pounds per acre, in which case larger amounts of aluminum introduced a small negative error. Inasmuch as all attempts to prevent aluminum interference by use of competitive complex formers or by increasing the alkalinity of the solution as suggested by Baver and Bruner (2) failed, the next obvious alternative, the method of compensation similar to that employed to prevent the interference of calcium, was tried and found quite satisfactory. The action of aluminum in the soil extracts is compensated for by the addition of aluminum to the standard solutions as well as to the soil extracts.

The interfering action of manganese was found to be more serious and complicated than that of calcium or aluminum. In the absence of magnesium, manganese gave no color lake with titan yellow but produced a brown coloration. With smaller amounts of magnesium, manganese appreciably reduced the color intensity or destroyed the magnesium lake, depending on the amount of manganese present. On the other hand, with larger amounts of magnesium, the effect of increasing the amount of manganese was to intensify the color of the magnesium lake. Thus, the interference of manganese rendered the result of the magnesium test very misleading in many instances. Certain acid soils containing large quantities of soluble manganese showed less than 25 pounds of magnesium per acre, whereas the examination of the same extracts by the 8-hydroxyquinoline method previously described (24) actually revealed over 100 pounds of magnesium, which may be considered adequate to meet the requirements of most crops. Another example may be cited to illustrate the magnitude of the errors introduced by manganese interference. In a teaching demonstration of cation-exchange phenomena, a number of soils containing appreciable

quantities of exchangeable manganese were extracted separately with water and an equal volume of 1 N potassium chloride solution. When examined by the titan yellow test, considerably more magnesium was found in the aqueous extracts than in the corresponding potassium chloride extracts, thus defeating, of course, the very object of the experiment.

The use of citrate, tartrate, pyrophosphate, and other differential complex formers to prevent manganese interference proved unsatisfactory, as they either caused precipitation of the lake or destroyed the color. Mannite was found effective in preventing the interference of only small amounts of manganese as previously reported (23). In comparing the influence of different reducing agents on manganese interference, the authors' attention was drawn to the interesting observation of Gillam (10) relative to the beneficial effect of hydroxyl-

TABLE 4

Effect of diverse ions on the proposed titan yellow test for magnesium

CONSTITUENT PRESENT	Mg FOUND	CONSTITUENT PRESENT	Mg FOUND
lbs./A.	*lbs./A.*	*lbs./A.*	*lbs./A.*
4000 Ca	0	400 Mg + 100 Al	400
50 Mg + 3800 Ca	50	400 Mg + 180 Al	350
100 Mg + 3200 Ca	100	100 Mg + 90 Fe^{+++}	120*
200 Mg + 3200 Ca	200	100 Mg + 90 Fe^{+++}	100
400 Mg + 1200 Ca	350	200 Mg + 90 Fe^{+++}	220*
400 Mg + 2400 Ca	350	200 Mg + 90 Fe^{+++}	200
200 Al	0	50 Mg + 190 Mn^{++}	?brown*
50 Mg + 100 Al	45	50 Mg + 190 Mn^{++}	65
50 Mg + 200 Al	45	100 Mg + 180 Mn^{++}	?brown*
100 Mg + 100 Al	100	100 Mg + 180 Mn^{++}	110
100 Mg + 200 Al	90	200 Mg + 160 Mn^{++}	110*
200 Mg + 100 Al	200	200 Mg + 160 Mn^{++}	180
200 Mg + 200 Al	175	100 Mg + 90 P (ortho)	100
		200 Mg + 80 P (ortho)	200

* Hydroxylamine hydrochloride omitted.

amine hydrochloride with the titan yellow method in preventing color fading which was found to occur in some instances. Inasmuch as manganese was not removed by the procedure employed by Gillam to separate iron and aluminum as the phosphates prior to the determination of magnesium by the titan yellow method, it was thought that the beneficial effect of hydroxylamine hydrochloride reported by this investigator might well have been attributed to the action of this reagent in preventing manganese interference rather than to any direct effect on color fading of the magnesium lake. This opinion was confirmed by subsequent experiments, which showed that hydroxylamine hydrochloride is without effect on color fading of the magnesium lake in the absence of manganese and that this reagent is very effective in preventing manganese interference with the titan yellow test for magnesium. The mechanism of the action of hydroxyl-amine hydrochloride in preventing or minimizing the interference of manganese

probably involves complex formation in addition to inhibiting atmospheric oxidation of manganous ion in alkaline solution, because other reducing agents tried were not found satisfactory.

Although iron in the amounts usually found in soil extracts does not interfere seriously with this test, its possible interfering action is also prevented in the presence of hydroxylamine hydrochloride. Phosphate ion in the amounts found in soil extracts will not interfere. The results of magnesium determinations as obtained by the proposed titan yellow procedure in the presence of several diverse ions are listed in table 4. Despite the significant improvements made in this test, the recovery of magnesium from standard synthetic soil solution as shown in table 2 is not so satisfactory as the recoveries of other constituents.

p-Nitrobenzeneazoresorcinol test. Since it was impossible by any simple procedure to prevent manganese interference with the magnesium test employing *p*-nitrobenzeneazoresorcinol reagent, this test was not fully investigated. The following comments, however, should be made. The *p*-nitrobenzeneazoresorcinol test for magnesium is apparently subject to the same interferences listed under the titan yellow test, except that calcium interference with the *p*-nitrobenzeneazoresorcinol test is less serious and can be prevented by the addition of mannite as provided in the procedure. The presence of mannite also serves to inhibit rapid color fading, which otherwise was found very troublesome. Obviously, the *p*-nitrobenzeneazoresorcinol test for magnesium as herein described is not applicable to soil extracts containing appreciable amounts of manganese, iron, and aluminum but should be well suited for the rapid determination of magnesium in plant tissue extracts, for which purpose it has been successfully employed in this laboratory.

Determination of potassium

Largely because of its rather extensive use, the turbidimetric cobaltinitrite test for potassium has been studied perhaps more than any other rapid microchemical soil test, yet the difficulties encountered with some of the present techniques were equally as great. Although some of the rapid soil tests for potassium in current use gave excellent results with pure potassium chloride solutions, the results obtained on synthetic solutions and soil extracts were not only erratic but subject to large analytical errors. Aside from variability in the composition of the precipitate due to possible substitution of potassium by sodium and ammonium ions, the apparent turbidity is markedly influenced by particle size and crystal form of the precipitate as has been observed by Kime.[5] Thus, in addition to the amount of precipitate or the completeness of precipitation, both the particle size and the crystal form of the potassium-sodium cobaltinitrite precipitate also markedly influence the apparent turbidity, the point that has been very frequently overlooked in studies of the method reported in the literature (4, 17). It was not surprising, therefore, to find that the pres-

[5] Kime, C. D., Jr. Factors affecting the reproducibility attainable in the cobaltinitrite turbidimetric method for determining potassium. 1940. (Unpublished master's thesis. Copy on file Univ. Fla., Gainesville.)

ence of diverse ions which do not necessarily react or form a precipitate with the reagent should affect the final turbidity.

The first procedure investigated was essentially the one which was originally proposed by Bray (3) and which is now in rather extensive use. The test was conducted as follows: To 2 ml. of the extract in a vial, 0.3 ml. of 37 per cent solution of formaldehyde and 0.3 ml. of Bennett's cobaltinitrite reagent (as given by Bray) were added and thoroughly mixed; 2 ml. of 95 per cent ethyl alcohol was then carefully added down the side of the vial to form a layer on top of the solution, and the contents were mixed rapidly and uniformly by swirling the vial for 30 seconds. After 15 minutes the turbidity of the test solution was compared with that of a series of standard potassium solutions carried simultaneously through the same procedure. Despite this method of turbidity comparison against a series of standards, it was observed that certain soil extracts gave

TABLE 5

Results obtained by the potassium turbidimetric test involving the use of Bennett's cobaltinitrite reagent and ethyl alcohol, showing the anomalous effect of temperature in the presence of diverse ions

SOLUTION*	K			
	at 26°C.		at 20°C.	
	15 min.	45 min.	15 min.	45 min.
	lbs./A.	*lbs./A.*	*lbs./A.*	*lbs./A.*
Standard synthetic soil solution No. 1............	225	225	175	175
Standard synthetic soil solution No. 2............	200	200	125	125
Soil extract A....................................	125	150	75	90
Soil extract B....................................	75	75	75	75
Soil extract C....................................	25	25	25	25

* Standard synthetic soil solution No. 1 contained the same constituents as the standard solution given in table 2 except that ammonium, manganese, ferric, and aluminum sulfates were omitted; standard solution No. 2 is identical with that shown in table 2. The theoretical amount of potassium in both solutions was equivalent to 220 pounds K per acre.

consistently lower results with decrease in temperature, and that, unless the temperature remained constant, the results obtained on the same extracts would vary appreciably from day to day. This anomalous effect of temperature on turbidity development observed in many soil extracts was confirmed by results obtained with standard synthetic solutions as shown in table 5. It was also found that in such extracts, particularly at temperatures between 20° and 25°C., the turbidity, when compared with a series of standards, would often increase with time so that the amount of potassium found after 45 minutes would be consideraby higher than that found after 15 minutes. Although the influence of temperature in the cobaltinitrite test for potassium has long been recognized, it has been assumed heretofore that the temperature effect is independent of the composition of the test solution and may be compensated for by use of natural standards carried simultaneously through the procedure. Inasmuch as the sensitivity of the test to small amounts of potassium increases with

decrease in temperature, there has been a tendency to favor low precipitation temperatures, which are likely to lead to lower recoveries of potassium than those reported in table 5. In fact, Burkhart (4), on the basis of the results obtained with potassium chloride solutions, recommended that the temperature be maintained at 10° C. in order to increase the sensitivity of the cobaltinitrite test to small amounts of potassium.

Although fairly satisfactory results could be obtained by using Bray's procedure, as described above, between 28° and 30° C., this narrow temperature range is apparently too near the critical temperature above which the sensitivity is low and the results are likely to be erratic. Several methods of mixing the solutions after the addition of alcohol, such as motor stirring, stoppering and inverting the vial, were then tried, and although the necessity of rapid and uniform mixing as emphasized by Hance (11) and Kime⁵ was confirmed, the different methods of mixing failed to obviate the anomalous effect of temperature on turbidity development in the presence of diverse ions. This led to a study of other variables in the procedure, such as the concentration of sodium cobaltinitrite, the concentration of sodium nitrite, the kind and amount of alcohol, and finally to the development of the test herein described. It will suffice to say that the major difficulties were eliminated by substituting isopropyl alcohol for ethyl alcohol despite the fact that the latter produces greater turbidity and consequently renders the test more sensitive. In order to increase the sensitivity of the test with isopropyl alcohol, it was necessary to increase the amount of sodium cobaltinitrite, the amount of sodium nitrite, as well as the ratio of sodium nitrite to sodium cobaltinitrite. Although the addition of 1 ml. of the new reagent introduces considerably more sodium cobaltinitrite, the color of the test solution is less intense and consequently less troublesome in comparison of turbidity than that in the case of the former test, because of the higher concentration of sodium nitrite, which was found to reduce markedly the color intensity of the sodium cobaltinitrite in solution. The new procedure has been found to give very reproducible and accurate results in the presence of diverse ions over a wide range of temperature (see table 2).

Reference is frequently made to the effect that ammonia, except when present in large amounts, does not interfere with the cobaltinitrite test for potassium. In order to obtain more specific information on this point, experiments were set up to study the behavior of the ammonium ion in the absence as well as in the presence of formaldehyde, which is commonly employed in this test for the purpose of preventing the interference of ammonia. The procedure given under the proposed potassium test was followed except, of course, in the absence of formaldehyde, in which case the formaldehyde solution was omitted from the test solutions as well as from the corresponding series of standards. It will be noted from the results presented in table 6 that the magnitude of the error caused by the interference of the ammonium ion in the absence of formaldehyde is determined by the concentration of both the ammonium and the potassium ions present, and that formaldehyde, because of its capacity to form hexamethylene tetramine with ammonia, is a very effective agent in preventing the interfering

action of ammonia. In the absence of formaldehyde, even small amounts of ammonia will cause appreciable errors when large amounts of potassium are present. Upon addition of 0.3 ml. of 37 per cent solution of formaldehyde, as much as 1000 pounds of ammonium nitrogen per acre will not introduce a significant error regardless of the amount of potassium present. The behavior of the ammonium ion with and without the addition of formaldehyde in the proposed test, as shown in table 6, is essentially the same as that found in the former test involving the use of Bennett's reagent and ethyl alcohol.

As already emphasized, it is imperative that the final mixing of the alcohol layer with the test solution containing cobaltinitrite be done rapidly and uniformly. The use of mechanical mixing devices such as proposed by Hance (11)

TABLE 6

Effect of the ammonium ion* on the proposed turbidimetric cobaltinitrite test for potassium in the absence and in the presence of formaldehyde

IN THE ABSENCE OF $HCHO$			IN THE PRESENCE OF $HCHO$		
Amount present		K found	Amount present		K found
K	NH_2-N		K	NH_3-N	
lbs./A.	*lbs./A.*	*lbs./A.*	*lbs./A.*	*lbs./A.*	*lbs./A.*
0	100	0	0	600	0
0	200	25	0	1200	0
0	300	50	0	2000	0
0	400	>300	0	2000	>300†
50	50	60	0	2000	>300†
100	50	110	100	100	100
200	50	225	200	100	200
50	100	75	100	300	100
100	100	125	200	300	200
200	100	240	100	600	100
50	200	125	200	600	225
100	200	200	100	1200	100
200	200	>300	200	·1200	225

* Ammonium added as the sulfate.
† 0.1 ml. of HCHO added instead of 0.3 ml.

and Kime[5] should permit more rigid standardization of this important step in the test. It is possible, however, for most operators to become proficient with hand mixing as employed in the proposed test after several days of practice.

Determination of manganese

The delicate benzidine spot test for manganese, proposed by Feigl (8) and which is commonly employed in rapid microchemical soil tests, was found unsatisfactory because the color fades entirely too rapidly to permit any quantitative estimation of manganese. In the formaldoxime method described by Sideris (28), iron and phosphate interfere and must be removed. As suggested by the recent polarographic investigations of Kolthoff and Watters (14), attempts

were made to oxidize manganese with lead dioxide to the trivalent state to form a violet complex, tridihydrogen pyrophosphatomanganiate, which is much more stable than the permanganate ion toward reducing agents, but the method was not found sufficiently sensitive for the purpose.

Of the colorimetric methods based on the oxidation of bivalent manganese to permanganate, the bismuthate method proved the most satisfactory. The oxidation of manganese proceeds very rapidly at room temperatures, and the permanganate color is fairly stable in acetate solutions in the presence of 0.2 ml. of concentrated sulfuric acid as given in the procedure. Rapid fading of color was observed with larger amounts of sulfuric acid, and also when nitric acid, which is commonly employed in the bismuthate method, was substituted for sulfuric acid. As in other methods involving oxidation of manganese to permanganate, all reducing substances must be absent. For this reason, the bismuthate test for manganese cannot be carried out successfully even in the presence of small amounts of organic matter in the extracts. Soil extracts of mineral soils, prepared without the use of activated carbon to remove soluble organic matter, gave no perceptible color in the bismuthate test although the extracts were known to contain an equivalent of at least 100 pounds of manganese per acre. This source of interference is virtually eliminated, however, when the extracts are clarified by means of activated carbon, under which condition the test may be expected to give reliable results. Because of the possibility of slight color fading on standing, the comparisons should be made as soon as the excess bismuthate has settled out, which usually takes about 30 minutes.

Determination of iron

Although o-phenanthroline (9) and $\alpha\alpha'$-bipyridyl (22) have been shown to be excellent reagents and superior to ferrocyanide or thiocyanate for the colorimetric determination of iron, these two reagents have received relatively little attention in rapid chemical soil testing work. Both reagents are highly specific and very sensitive to ferrous iron with which they form very stable colored complexes over a wide range in pH values (pH 2 to 9). There is little choice between the two reagents. When stoppered and protected from direct light, the standards may be kept for 6 months without any color fading (9).

The adoption of the o-phenanthroline reagent as a rapid microchemical soil test for iron in soil extracts presented no problem. Nevertheless, several questionable points required further consideration. Tests made to determine the rate of reduction of ferric iron by hydroxylamine hydrochloride showed that even at pH 4.8, under conditions of the test, the reduction was complete within 1 minute. The addition of o-phenanthroline before or after the addition of hydroxylamine hydrochloride to a solution of ferric iron gave the same results. In the absence of the reducing agent, o-phenanthroline produced a barely perceptible yellowish coloration with ferric ion. Thus it should be possible by means of this reagent to determine the relative amounts of both ferrous and ferric ions in the same solution by measuring the color intensity before and after reduction.

Determination of aluminum

Three of the organic reagents commonly employed in the rapid microchemical soil tests for aluminum are hematoxylin, alizarin S, and aluminon (ammonium salt of aurintricarboxylic acid). Iron interferes seriously with the methods using the above reagents, and in the more accurate procedures it usually is removed by cupferron or sodium hydroxide separation. As the color produced with iron differs from that given by aluminum, accurate color comparison is impossible even in the presence of relatively small amounts of iron. With the exception of the procedure given by Baver and Bruner (2), no provision is made to prevent iron interference in the rapid microchemical soil tests for aluminum reported in the literature.

Comparison of the applicability of hematoxylin, alizarin S, and aluminon to the rapid estimation of aluminum in soil extracts showed the aluminon reagent to be superior in many respects. The aluminon method was found simpler and more sensitive over a wider range of aluminum concentration, especially in the presence of a protective colloid. It was also found possible to prevent the interference of iron with this method by means of hydroxylamine hydrochloride without resorting to tedious analytical separations. Upon addition of hydroxylamine hydrochloride, excellent recoveries of aluminum were obtained even in the presence of larger amounts of iron than are normally found in soil extracts. Comparative tests of the relative effectiveness of hydroxylamine hydrochloride and sodium sulfide, the reagent employed by Baver and Bruner (2) to prevent iron interference, showed hydroxylamine hydrochloride to be superior, as the use of sodium sulfide is likely to cause troublesome turbidities.

Unlike the intense color of the alizarin dye, the color of the aluminon reagent is hardly perceptible and the excess of the dye need not be decolorized with ammonium carbonate, as is commonly done, especially when color comparison is made against a series of standards. Even when other methods of color comparison are employed, such as light transmission measurements by means of a photoelectric colorimeter, the excess dye should not be decolorized with ammonium hydroxide or ammonium carbonate when hydroxylamine hydrochloride is used to prevent the interference of iron, because under such conditions very rapid color fading has been observed. The presence of other reducing agents such as hydrogen sulfide and sulfur dioxide has also been reported to destroy the aluminum lake (36) presumably under the same conditions. It is very improbable that such metals as chromium, thorium, and a number of the rare earth metals, the hydroxides or basic acetates of which also form red lakes with aluminon but which are decolorized by ammonium carbonate (19), are present in sufficient amounts in soil extracts to cause significant errors.

The precipitation of the lake not only limited the useful working range of the test but made accurate color comparison very difficult. This difficulty has been obviated by the fivefold dilution of the extract and by the use of soluble starch. Fortunately, the same protective colloid may be employed to advantage here as well as in the magnesium test. The pH of the soil extracting solution employed, i.e., 4.8, is optimum for the development of the maximum color in-

tensity (35), and because the solution is strongly buffered at this pH value, further adjustment of the pH of the soil extracts in making the aluminum test is unnecessary. Although the aluminum lake develops slowly at room temperatures and the full color is not attained within 24 hours, the comparison with a series of standards prepared simultaneously may be made at any time after 30 minutes. The results of aluminum determinations as obtained by the proposed test in the presence of diverse ions may be found in table 2.

Determination of phosphorus

The rapid microchemical soil tests for phosphorus are modifications of the well-known method involving the formation of phosphomolybdic acid in acid solution and the subsequent selective reduction of the heteropoly acid with stannous ion to give molybdenum blue. Examination of the conditions under which some of the tests are made would indicate that the influence of such variables as the nature and the concentration of the acid employed, the amount of molybdate, the ratio of molybdate to acid, and the amount of reducing agent is not fully appreciated. The acidity in some of the procedures has been reduced below the critical point at which silica interferes and the molybdate itself is likely to be partly reduced to give the blue color in the absence of phosphate. Also, the use of powdered stannous oxalate or tinfoil, as commonly employed, offers little advantage and is not recommended, as such techniques have been found to give less reproducible results than the addition of stannous chloride solution, which is reasonably stable when protected from air with a layer of paraffin oil. Unfortunately, other procedures that utilize more stable reagents and also give more stable molybdenum blue color are not sufficiently sensitive to permit their use on soil extracts very low in phosphorus, such as those obtained with Morgan's extracting solution.

The phosphorus test described here is carried out in the presence of a high concentration of hydrochloric acid, under which condition silica and relatively large amounts of ferric iron as shown by Dickman and Bray (7) do not interfere. No interference was observed from ferric iron in amounts equivalent to 100 pounds per acre. Larger amounts of ferric iron decreased the intensity of the color, but it was found that the interference from as much as 300 pounds of ferric iron per acre could be prevented by the addition of three drops of a 5 per cent solution of hydroxylamine hydrochloride to the test solution prior to the addition of the molybdate reagent. Nitrate does not interfere. Arsenate, of course, reacts like phosphate under the same conditions and must be absent. Its interfering action, however, may be eliminated upon reduction with sodium bisulfite in hot acidified solution before the addition of molybdate (37). Attempts to reduce pentavalent arsenic with other reducing agents at room temperature and without further addition of acid to the extracts were not successful. Silica will not interfere, provided the molybdate reagent is added rapidly and mixed immediately with the soil extract in order to prevent the formation of silico-molybdate. Failure to observe this precaution may lead to serious errors.

If the pH of the soil extract is above 5.2, which is seldom the case except

with highly calcareous soils, the amount of acid provided in the molybdate reagent may be insufficient to prevent some reduction of the uncombined molybdate. In such cases, the extract should be diluted with the extracting solution, or the pH adjusted to 4.8 with hydrochloric acid, before proceeding with the test.

Determination of ammonia

Preliminary trials to nesslerize the ammonia directly in the soil extracts in accordance with the procedure followed in all rapid microchemical soil tests were unsuccessful because of precipitation of the Nessler reagent in the presence of the high concentration of sodium acetate and other diverse ions, notably magnesium. The formation of red precipitates immediately upon addition of the Nessler reagent to the extracts, even in the absence of ammonia, greatly impaired the accuracy of this delicate test and made the color comparison very difficult or impossible. These difficulties due to precipitation of the Nessler reagent and the colored ammonia complex have been completely obviated by the use of tartrate and gum acacia, the latter serving as a protective colloid. The purpose of the tartrate is to prevent the precipitation of certain ions, particularly magnesium, which even in small amounts was also found to cause rapid precipitation of the color in nesslerized solutions. With this procedure, it has been possible to make direct nesslerization of relatively large amounts of ammonia in soil extracts in the presence of diverse ions without the formation of precipitates in nesslerized solutions even upon standing for several hours.

The Nessler reagent is prepared according to the directions given by Vanselow (31) except that it is twice as concentrated. This reagent is more readily prepared and has been found more satisfactory for direct nesslerization than other modifications in common use. Purification of unground gum acacia, U. S. P., has been found unnecessary, but its slight reducing action on the Nessler reagent must be destroyed by pretreatment of the gum acacia solution with Nessler reagent as recommended by Chiles (5). The reagent prepared by combining the Nessler reagent, gum acacia, sodium hydroxide, and sodium tartrate proved unsatisfactory because of rapid deterioration.

To prevent possible loss of ammonia, it is necessary to add Nessler reagent soon after mixing the extract with the sodium hydroxide-tartrate solution; however, the completion of the test may be delayed even 1 hour without loss of ammonia, provided the sodium hydroxide-tartrate solution is added down the side of the vial as directed in the procedure.

The procedure for direct nesslerization herein described has been also successfully employed for the determination of the exchange capacity of soils by the ammonium acetate method, thus supplanting the distillation of ammonia subsequent to its extraction with sodium chloride solution as previously described (24).

Determination of nitrate

Among the three reagents, diphenylamine, β-methylumbelliferone, and brucine, which were investigated by the authors, brucine was found superior to

either of the others for the rapid determination of nitrate in soil extracts. The color produced by β-methylumbelliferone with nitrate in strong sulfuric acid solution was not sufficiently intense, and though the sensitivity of the test could be increased by subsequent neutralization of the solution with ammonium hydroxide as proposed by Vasil'ev and Dukhinova (32), this procedure obviously lacks the desired simplicity. Although equally simple and rapid, the accuracy as well as the precision of the brucine test was found to be much greater than that of the diphenylamine spot plate test which was originally proposed by Morgan (20) and which is now extensively employed for rapid estimation of nitrate in both soil and plant tissue extracts. Moreover, the brucine test is remarkably free of interferences by extraneous ions. In fact, none of the ions in the soil extracts are likely to be present in sufficient quantities to interfere with this test. It is surprising, therefore, that with the exception of the test for nitrate employed by Truog[3], this reagent has found no application in the rapid microchemical soil tests. Truog's procedure, however, makes use of the initial bright red color which appears in the presence of nitrate but which changes rapidly to a transitional orange and finally to a permanent yellow color. This permanent yellow color as employed in the proposed test is more reliable and permits more accurate color comparison with a series of natural standards.

A few tests made to study the behavior of nitrite showed that, under the specific conditions of the test, nitrite is apparently also determined and included together with nitrate nitrogen in the final result. In order to check the accuracy of the proposed brucine test, nitrate determinations on soils shown in table 7 were also made by the phenoldisulfonic acid method as described by Harper (12) except that a photoelectric colorimeter was employed to measure the light transmission. It is evident from the results in table 7 that the agreement between the two methods is very satisfactory.

<center>COMMENTS</center>

Recoveries of added constituents from a synthetic soil solution as obtained by the proposed microchemical soil tests are shown in table 2, which has already been referred to in the discussion. Additional data relative to accuracy and sensitivity, as well as some information on the completeness of extraction of the exchangeable bases, are given in table 7, in which the results of the proposed microchemical soil tests on some typical New York soils are compiled together with other data obtained by the more conventional methods.

Because of the slow deterioration with time of some of the reagents and the influence of temperature and time in certain tests, the use of artificial standards, such as color and turbidity charts, is likely to lead to appreciable errors. The photoelectric colorimeter, which is now rapidly supplanting the visual methods of color comparison, offers little advantage, as it is subject to the same limitations. Instead of resorting to temperature control and timing, both of which are impractical when dealing with batteries of 24 to 36 soils at a time, it is more expedient and feasible to employ natural standards. When making tests subject to the time factor on this many soil extracts, however, it is advisable to develop the color or turbidity in the standards after the color or turbidity has been

TABLE 7

Comparison of results of the proposed rapid microchemical soil tests with those obtained by the more conventional methods

Acre in terms of 2,000,000 pounds

SOIL TYPE, DEPTH 0-6 INCHES	pH	RESULTS OF RAPID MICROCHEMICAL SOIL TESTS, LBS./A. P	NO₃-N	NH₃-N	Ca	Mg	K	Mn	Fe	Al	EXCHANGE CAPACITY M.E./100 GM.*	EXCHANGEABLE CATIONS,* LBS./A. Ca	Mg	K	Mn	ACID-SOLUBLE P,† LBS./A.	NO₃-N, LBS./A. (PHENOLDISULFONIC ACID METHOD)‡	RATIOS OF CATIONS Ca	Mg	K	Mn
Collamer silt loam	5.65	3	10	45	2400	250	55	60	8	60	12.8	2820	355	82	17	40	8	0.85	0.70	0.67	3.53
Coloma fine sand	4.55	6	275	115	1000	250	70	80	12	200	7.20	1160	66	83	51	105	275	0.87	0.38	0.84	1.57
Coloma fine sand	4.55	7	250	125	1000	25	75	140	18	200	9.25	1350	64	110	145	105	250	0.74	0.39	0.68	0.97
Coloma fine sand	4.35	6	350	50	800	25	75	85	30	360	9.05	1020	50	120	41	60	330	0.78	0.50	0.63	2.07
Dunkirk loam	5.40	5	8	70	1400	50	50	110	20	100	7.55	1630	90	47	8		3	0.86	0.56	1.06	13.7
Gloucester loam	6.62	5	20	35	2200	250	60	30	3	75	10.1	2550	370	253	2	90	20	0.87	0.68	0.99	15.0
Gloucester loam	6.12	3	2	40	2200	30	110	30	8	230	9.70	2370	77	110	3	10	2	0.93	0.39	1.00	10.0
Gloucester gravelly loam	6.02	22	2	35	2200	360	100	55	12	85	13.7	2740	535	115	7	203	2	0.80	0.67	0.87	7.85
Honeoye silty clay loam	6.00	8	110	140	2800	330	200	150	2	15	11.5	3040	520	263	43	80	95	0.92	0.63	0.76	3.49
Honeoye silty clay loam	5.50	6	100	80	1800	250	140	150	5	55	10.6	2230	396	196	115	70	100	0.77	0.63	0.71	1.30
Honeoye silty clay loam	6.52	3	5	30	4000	450	75	120	1	10	16.0	4280	750	123	10	28	6	0.93	0.60	0.61	17.1
Honeoye silt loam	7.00	20	70	45	5000	480	125	85	1	5	17.0	5770	750	146	10	135	66	0.87	0.64	0.86	8.50
Honeoye loam	7.38	25	25	50	5000	675	175	140	2	15	8.80	2280	700	205	25	100	25	2.19	0.96	0.85	23.3
Hoosic sand	6.37	15	5	35	1800	35	150	35	5	65	4.70	1500	45	147	5	60	6	1.20	0.78	1.02	7.00
Hoosic gravelly loam	5.62	5	5	60	1800	60	125	60	8	160	13.3	2080	132	131	12	57	7	0.86	0.45	0.95	5.00
Mardin silt loam	6.10	6	10	60	5000	30	100	40	15	120	17.0	4850	88	120	7	40	5	1.03	0.34	0.83	5.70
Mardin silt loam	4.77	4	140	90	2000	70	160	150	35	240	17.9	2340	163	218	78	85	150	0.85	0.43	0.73	1.92
Merrimac fine sandy loam	5.32	4	2	15	400	55	60	50	10	200	6.16	290	77	67	5		1	1.38	0.71	0.90	10.0
Ondawa loam	5.67	10	12	50	2000	100	70	120	7	70	10.3	2350	162	77	17	83	14	0.85	0.62	0.91	7.07
Palmyra shaly loam	4.78	6	25	80	1400	40	170	70	55	280	14.7	1585	114	200	25	50	24	0.88	0.35	0.85	2.80
Palmyra shaly loam	5.43	7	120	65	3700	75	175	125	10	90	18.9	4560	147	200	34	15	105	0.81	0.51	0.88	3.68
Volusia silt loam	5.15	1	5	65	2000	55	160	150	30	180	15.4	2440	118	188	12	11	7	0.82	0.47	0.85	4.17
Volusia silt loam	4.97	1	8	55	1600	75	70	150	60	200	15.6	1920	198	103	20	63	4	0.83	0.38	0.68	7.50
Volusia silt loam	6.27	6	4	40	3500	110	80	70	2	30	14.6	4450	224	130	4		3	0.79	0.49	0.62	17.5

* Exchange capacity and exchangeable cations by the ammonium acetate method as described by Peech (24).

† Phosphorus soluble in Truog's 0.002 N H₂SO₄ solution at 1:100 dilution.

‡ Nitrate nitrogen by phenoldisulfonic acid method as described by Harper (12).

developed in one half of the extracts before completing the tests on the remainder of the extracts. With this precaution, the relatively small differences in time of color or turbidity development between the soil extracts and the standards will introduce only negligible errors.

The microchemical soil tests herein described should be applicable to the examination of aqueous extracts and soil solutions, particularly when dealing with limited volumes of soil solutions as obtained by the pressure-membrane technique of Richards (26), and when the degree of accuracy obtainable by such micromethods as those described by Reitemeier (25) or Peech (24) is not required. In order to employ advantageously the same reagents and standard solutions listed under the respective tests without appropriate modifications, the soil solution or the aqueous extract, subsequent to concentration or any preliminary treatment to remove the organic matter, should be made to contain the same concentrations of sodium acetate and acetic acid as are present in Morgan's extracting solution. In the removal of organic matter from soil solutions by carbon, Reitemeier (25) found appreciable reduction in concentration of many ions. There is no apparent reason, however, why activated carbon should not serve the purpose equally well here after the addition of proper amounts of sodium acetate and acetic acid to the soil solution or the aqueous extract, under which conditions adsorption of most ions by carbon, as has been already shown, is negligible.

Attempts to adapt these microchemical soil tests to the determination of soluble plant nutrients, after extraction of fresh plant tissue with Morgan's extracting solution in a Waring Blendor, have shown that the tests for ammonia and nitrate nitrogen are not satisfactory for this purpose. Although perfectly clear and colorless plant tissue extracts could be obtained by the use of activated carbon, some of the extracts were found to contain interfering organic substances, presumably sucrose and reducing sugars, which reduced the mercuric ion in the Nessler reagent and which also interfered seriously with the nitrate test. The addition of sulfuric acid to such extracts even in the absence of brucine produced an intense yellow to brown coloration, thus rendering the test worthless. Obviously, other nitrate tests, employing reagents such as diphenylamine, β-methylumbelliferone, and phenoldisulfonic acid in sulfuric acid solution, are subject to the same interference. As diphenylamine gives a blue color with nitrate, the interference with this test is probably less serious. Although the tests for other constituents apparently gave good results on plant tissue extracts clarified with activated carbon, possible interferences with other tests merit further investigation.

SUMMARY

A critical examination of the rapid microchemical soil tests in current use showed many of the tests to be subject to serious analytical errors and, therefore, unreliable. Among some of the inherent technical difficulties, interferences by diverse ions were found to cause sufficiently serious errors to invalidate the results in many instances. These interferences in the tests were thoroughly investigated and were obviated by the development or the adoption of more specific reagents and by use of competitive complex formers. The accuracy

as well as the working range of the tests, particularly those utilizing color lake formation, was considerably increased by introduction of protective colloids. As a result, accurate and reliable microchemical soil tests suitable for practical routine soil testing have been developed. These are presented in this paper together with a discussion of interferences and other inherent analytical errors encountered in the course of the work. The applicability of these tests to the examination of soil solutions, aqueous extracts, and plant tissue extracts is also discussed. A brief description of the tests follows.

The soil is extracted with Morgan's sodium acetate-acetic acid solution at pH 4.8. The removal of soluble organic matter is effected by the use of activated carbon in the extraction process. All tests are then made directly on the separate aliquots of the filtered extracts without preliminary separation or treatment. The amounts of the different constituents are determined by comparison with a series of standard solutions carried simultaneously through the tests. Calcium is estimated turbidimetrically as calcium stearate; magnesium, colorimetrically by means of titan yellow; potassium, turbidimetrically as potassium-sodium cobaltinitrite; manganese, colorimetrically after oxidation with bismuthate; iron, colorimetrically by o-phenanthroline; aluminum, colorimetrically by aluminon (aurintricarboxylic acid); phosphate, colorimetrically after reduction of the phosphomolybdate with stannous oxalate; ammonia, colorimetrically by direct nesslerization; and nitrate, colorimetrically by brucine.

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LABORATORY PERCOLATION THROUGH UNDISTURBED SOIL SAMPLES IN RELATION TO PORE-SIZE DISTRIBUTION[1]

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Whether soil water moves by turbulent flow through large root channels, by streamlined flow between natural structural units, or by film adjustments in the finest capillaries, its movement is related to the size and arrangement of the pores. Once the natural arrangement is disturbed in the laboratory there is no way of reconstructing it. Measurements of texture and aggregation may be of assistance, but they are not adequate to indicate the true conditions of permeability and aeration in the original soil mass.

There are methods, however, that offer promise as means of evaluating pore-space relationships in undisturbed soil samples. These are based upon direct measurements of permeability to water or gases (1, 6, 11, 16, 19, 21, 26, 28, 31) and upon determinations of effective pore-size distribution by moisture tension methods (1, 5, 8, 14, 15, 16, 25). Direct permeability measures have received widespread consideration both in soils and in hydrologic studies for many years, and numerous mathematical expressions have been worked out relating percolation to particle size and pore volume. The classic equation is that of Schlichter, which is cited in its original or modified form in almost every text dealing with water movement. This equation, as discussed by Zunker (30, 31), was developed theoretically on the basis of Poiseuille's law for viscous flow (7), and has been widely used in studies of underground waters.

The validity of such a formula for estimating ground-water movement is dependent upon a dominance of lamellar or viscous flow and the soundness of Darcy's law, as well as upon the accuracy of the evaluation of pore size, shape, and continuity.

The thorough discussion of lamellar flow by Tolman (29) seems to indicate that most ground-water movement is of this nature, and the precise experimental

results of Meinzer (17) provide a basis for concluding that Darcy's law is valid for all practical purposes. The same conclusion is reached by Muskat (20). But, even so, it is evident that such formulas can never be used to evaluate accurately the movement of water through soils. No means is provided for expressing differences in pore continuity, and the pore size is only very roughly approximated by the best possible measurements of particle size. Differences in the packing of soil particles, which are reflected in the conclusion of Bodman (4) that very little water moves through soils finer than fine sandy loams at apparent densities greater than about 1.5, could be accounted for only to a very limited extent by the porosity and particle-size factors in Schlichter's formula.

Somewhat improved accuracy in predicting percolation through soils might be expected from the formula of Zunker (30, 31), which distinguishes between effective and noneffective pores upon the basis of specific surface or hygroscopicity of the soil.[3] This distinction, though arbitrary, is a step in the direction of attaching greater significance to the coarser pores. It accomplishes essentially the same thing as the division of soil pores into capillary and noncapillary pores on the basis of the moisture equivalent, a distinction which has provided a useful index to water movement (11, 19).

Despite the usefulness of such indexes, it is apparent that they are not entirely satisfactory (11, 19, 28). This has led to studies of water removal at low tensions in the hope of finding improved relationships (1, 5, 14, 16, 22, 25). One simple index with promise is the water removal by a 40-cm. tension suggested by Nelson and Baver (22). This places much greater emphasis upon the coarser pores than the Zunker formula or noncapillary porosity derived from the moisture equivalent and volume weight. The index does not entirely satisfy Nelson and Baver, however, as shown by the fact that they emphasize the need for further study. Baver (3) in a recent discussion re-emphasized the need for a closer evaluation of the effectiveness of the pores of different sizes and the need for more study of pore continuity.

Because of differences in directness and continuity of the pores, it is not to be expected that a perfect relation can ever be found between pore size and percolation even in the laboratory. This is indicated by Baver (3) and is brought out clearly by the results of Slater and Byers (26). Even so, there is an obvious need for more information about the relations that do exist in natural soil between percolation and pore size based on moisture tension. Such information should help to show the extent to which pore size can serve as an index to water movement, as well as the limitations of the relationship. Once the facts are understood, it would appear that the two measurements could be used to supplement each other by affording an answer to many questions as to the momentary physical condition of a particular soil sample with respect to water movement. That is, percolation measurements can indicate the permeability of a particular material, and pore-size or tension determinations can show what pores are available to account for the measured water movement. These are the two

[3] Hygroscopicity is determined over 10 per cent H_2SO_4.

questions which require an answer in many studies before relative physical differences between soil samples can be shown.

Experience in this laboratory indicates that wide variations are to be expected between duplicate samples representing a particular soil or condition. This will in some cases limit the use of percolation and pore-size measurements, but the magnitude of the real differences between materials or conditions is frequently so great that close agreement among duplicate samples is not necessary to show the differences. And in any case, care in collecting representative samples and sufficient replication can be used to bring out such differences as do exist, provided the interest in small differences is great enough to justify the effort.

The purpose of this paper is to show what has been found regarding the relation of water movement to pore size as indicated by tension. The results given are for individual samples, some of which are collected as replicates from a particular material.

MATERIALS AND METHODS

In studying the distribution of the soil pores involved in percolation, it is possible to use very simple techniques, since only the coarser pores need be considered. It seemed likely that a tension of 100 cm. of water would be sufficient to drain all pores making important contributions to percolation (1, 14, 15, 18, 22), and the data obtained have confirmed this belief. This tension is within the range of the apparatus of Leamer and Shaw (15) using ordinary desk blotters. A similar technique was used in the present study except that individual cores in brass cylinders were clamped to a brass base through which various tensions could be applied by raising or lowering a rubber tube filled with water, as suggested by Donat (8). The brass base was previously fitted with a fine copper screen overlain by two layers of blotting paper which maintained saturated contact between the water column and the soil. This simple apparatus (fig. 1) has the advantage that the soil may be wetted, then quickly drained at the various tensions up to slightly more than 100 cm. of water without being removed from the base. It is especially convenient in obtaining accurate determinations at very low tensions and rates of water movement under various tensions.

Preliminary to the determination of percolation rates, the samples are allowed to wet by capillarity. They they are clamped to the brass base and a low hydraulic head is applied from below, causing water to move through the sample. Percolation rates were determined in two ways in this study: first, by downward percolation through the soil with a thin layer of water over the upper soil surface and a blotting paper membrane at the lower surface (fig. 1); and second, by percolating the water upward through the soils from below with no membrane of any kind except a layer of muslin (fig. 2). In both cases the sample was wetted from below as described.

For downward percolation (fig. 1) the water level was maintained by pouring on additional water at intervals to replace that moving down through the soil

and out into the graduate.   A fragment of blotting paper held on the water sur-
face served to dissipate the force of the water during pouring, thus preventing
any dispersion of the soil.   The fluctuation of the water level was controlled
within less than 0.5 cm., and a complete film of water was maintained over the
soil surface at all times.   The thickness of the water layer over the soil varied
somewhat, depending on the depth of the sample, but the total hydraulic head
was adjusted by means of the outlet tube so that it was equal to the depth of the
soil sample.
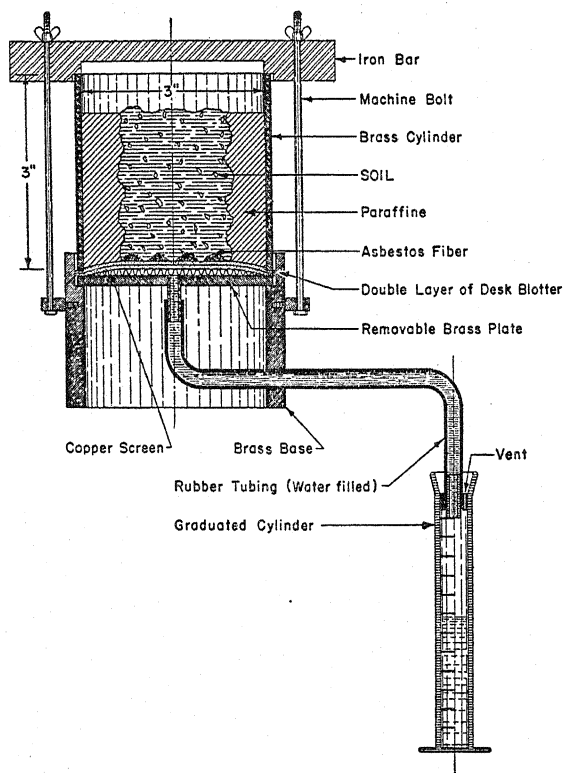
FIG. 1. APPARATUS FOR MEASURING PERCOLATION RATES AND MACROPOROSITY OF NATURAL
SOIL

During upward percolation (fig. 2) the water level was maintained equal to
the depth of the soil sample by means of automatic flow from a large reservoir
with a floating valve.   The maximum fluctuation of the hydraulic head was
approximately 0.5 cm.

The percolation rates are expressed in inches per hour, the units suggested by
Richards (24) as having the advantage of certain practical implications.   This
expression may be directly converted into units like the Darcy coefficient of
permeability, or simple the "darcy" as used by Muskat (20).[4]

[4] One inch per hour = 0.7 darcy.

Percolation from below was allowed to take place for a few minutes before the rate was measured by either method. Volumes of flow for suitable intervals are ordinarily constant within 10 per cent for successive readings. If the volume of percolate is sufficient for accurate measurement, the rate may be obtained in a few minutes. For samples with no coarse pores it is necessary to allow considerable time for wetting and for measuring the percolation rate.

Percolation rates can be measured with precision to 0.01 inch per hour and can be estimated to 0.001 inch per hour. The smaller absolute values are significant only when very slow percolation rates are being considered. Among very rapid rates a variation of 1.0 inch per hour may be of little interest.
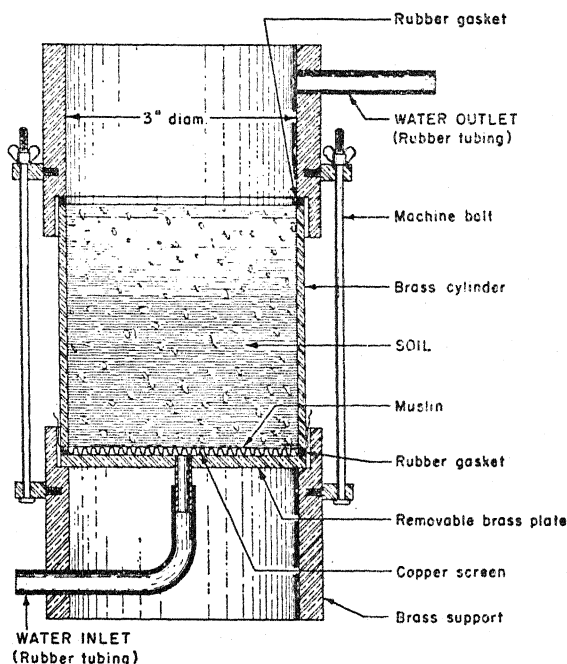

FIG. 2. APPARATUS FOR DETERMINING PERCOLATION RATES OF NATURAL SOIL CORES. FLOW NOT IMPEDED BY A MEMBRANE

Immediately after determining the percolation rate, water removed by tensions of 10, 40, and 100 cm. of water column was measured successively, in the apparatus described (fig. 1), by adjusting the height of the outlet tube to provide the desired tensions and measuring the water removed at each tension. It was necessary to replace the muslin (fig. 2) with a blotting paper membrane in those cases where only muslin was used during percolation.

The only appreciable error involved in determining the water removed by this means during a single determination is the reading of the volume of water in the graduate. This amounts to about 0.1 per cent in terms of the total soil volume, which is insignificant except for the measurement of the coarse pores in

samples with slow percolation rates.   In such cases, this 0.1 per cent represents the approximate limit in accuracy that can be expected for establishing any kind of relation between percolation and the volume of pores of a particular size.

If repeated determinations are made with a particular sample, there is likely to be a rather large variation, as has been shown by Russell (25).   He concluded that a part of this variation was due to structural changes within the soil sample and that the first set of tension results were at least as satisfactory for appraisal of soil differences as results obtained after repeated wetting.   In the present case also it was decided to use the results of the first tension measurements. This would seem most likely to yield results that would be typical of the natural soil as collected, because the structural changes of a core sample in the laboratory would not necessarily follow the same course as structural changes in the field.   And regardless of whether the results would apply directly to field conditions, the primary object of this study would be satisfied, that is, to establish any relationships that may exist between percolation rates and pore sizes based on tension.   In most cases the general relations are believed to be much more than momentary, but even if they were not, it would seem worthwhile to measure the effective pore sizes immediately following percolation so that the best possible indication would be obtained as to the pores that were permitting the actual percolation measured.

Collection and treatment of samples

Soil samples were collected at moisture contents near field capacity in a sampler 3 inches in diameter, described by Baver (1) as the Bradfield sampler, or as natural masses of soil, which will be referred to as lumps.   These lumps were collected from subsoils after it was found that cores from subsoils were often compressed enough by the sampling to alter completely their natural percolation rates and pore sizes.

In using soil lumps, the volume of each was determined by sand displacement (26) and a core for percolation and macroporosity measurements was prepared by sealing these lumps within 3-inch brass cylinders by means of molten paraffin. Fine asbestos fiber was used to fill any small irregularities of the lower soil surface which was to be brought into saturated contact with the blotting paper membrane.   This procedure has been described elsewhere (27).   A soil lump in the tension apparatus is shown in figure 1.   Complete cores differed only in that the cylinder was filled with soil, no paraffin being used.   The tension on the blotting-paper membrane and soil was adjusted by raising or lowering the water-filled tubing.   The minimum cross section of the lump was estimated with a suitable scale.   The error in such an estimation is naturally large, but the fact that percolation rates are invariably subject to a large percentage variation allows the use of this estimated cross section without any appreciable sacrifice in accuracy.

The soil samples were kept moist in sealed containers until the determinations were made.   A few drops of toluene were added to each sample to prevent fungus growths.

Nature of samples

The samples studied cover a rather wide range of soil characteristics. They varied in texture from sandy loam to clay. Some samples were from surface horizons; others were from subsurface soils and parent materials. The cores used were 3 inches in diameter by $1\frac{1}{2}$ to 3 inches long. Most samples were taken in permanent pastures, but a few were from cultivated areas and from timber land. A few laboratory-packed quartz sand separates were studied for comparison. The character of individual soil samples will be discussed in connection with the results.
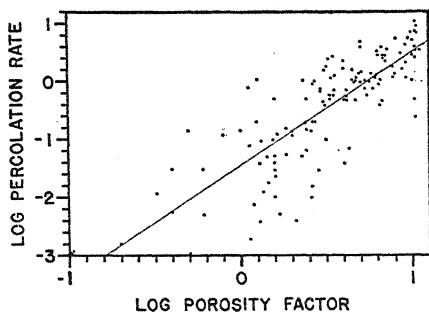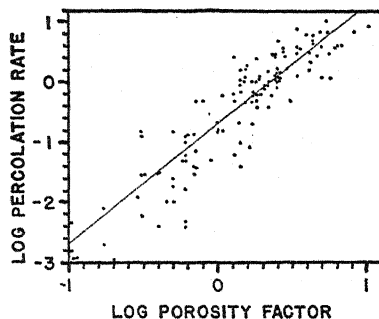

FIG. 3.   FIG. 4.

FIG. 3. RELATION BETWEEN PERCOLATION RATE AND PERCENTAGE POROSITY DRAINED BY A 40-CM. TENSION, FOR SAMPLES COLLECTED UNDER PERMANENT VEGETATION. PERCOLATION THROUGH A BLOTTING PAPER MEMBRANE

FIG. 4. RELATION BETWEEN PERCOLATION RATES AND A POROSITY FACTOR INVOLVING ONLY THE PORES DRAINED BY A 40-CM. TENSION, FOR SAMPLES UNDER PERMANENT VEGETATION. PERCOLATION THROUGH A BLOTTING PAPER MEMBRANE

Porosity factor = % pores drained by 10-cm. tension

$$+ \frac{\% \text{ pores drained from 10- to 40-cm. tension}}{4}$$

RESULTS

Noncultivated samples

As the first step in determining some interrelations between percolation rates and pore sizes, the percentage of pores[5] drained by a 40-cm. tension was considered, on the basis of the suggestion by Nelson and Baver (22) that pF = 1.6 seemed to give the most satisfactory single key to percolation rates for laboratory packed samples. All these percolation rates were determined downward through the samples with the blotting paper in place. No cultivated surface soils were included.

The relation is shown in figure 3, both the percolation and the porosity being plotted logarithmically to give a straight line regression. The regression equation is $Y = 1.95\,X - 1.44$, with a standard error of estimate of 0.523.

[5] Percentage of the total soil volume.

In order to test the extent to which an improved relation between percolation and pore size could be obtained by attaching greater significance to the coarser pores, a general porosity factor was adopted as follows: Porosity factor = % pores drained by a 10-cm. tension $+\dfrac{\text{\% pores drained between 10- and 40-cm. tensions.}}{N}$

When different even values of N were used, the closest relation was found at $N = 4$, as measured by the standard error of estimate of the log of the percolation rate and log of the porosity factor from a straight line regression. The standard error for $N = 2$ was 0.473; for $N = 4$, 0.461; for $N = 6$, 0.477. Figure 4 shows the relation between log percolation and log porosity factor with $N = 4$.

As a further test of whether an improved relation could be obtained by considering pores finer than those drained by a 40-cm. tension, attention was given to the pores from 40 to 100 cm. It was immediately obvious in considering
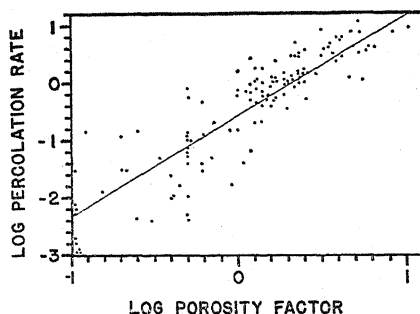

LOG POROSITY FACTOR

FIG. 5. RELATION BETWEEN PERCOLATION RATES AND A POROSITY FACTOR INVOLVING THE PORES DRAINED BY A 100-CM. TENSION, FOR SOIL SAMPLES COLLECTED UNDER PERMANENT VEGETATION. PERCOLATION THROUGH A BLOTTING PAPER MEMBRANE

Porosity factor = % pores drained by a 10-cm. tension
$+\dfrac{\text{\% drained between 10- and 40-cm. tension}}{4}$
$+\dfrac{\text{\% pores drained from 40- to 100-cm. tension}}{10}$

these that they must be given less weight than the pores from 10 to 40 cm., or the relation to percolation would lose accuracy. In order, therefore, to carry through approximately the same trend in effectiveness as that indicated for the coarser pores, the volume from 40 to 100 cm. was divided by 10 and this was added to the previous index, giving the porosity factor which will be referred to as

P.F. #1 = % pores to 10 cm. $+\dfrac{\text{\% pores from 10 to 40 cm.}}{4}$

$+\dfrac{\text{\% pores from 40 to 100 cm.}}{10}$

The relation, shown in figure 5 was an improvement, the standard error of estmate being 0.438 from the regression line $Y = 1.99X - 0.73$. The main effect was in giving some additional spread to the points representing low porosity values and low percolation rates.

No attempt was made to determine mathematically whether the divisor of 10 for the third term was the most nearly accurate value that could be used. It was clear from plotting the points that neither a divisor of 5 nor one of 20 gave as reliable a factor as the divisor of 10. The relatively small weight of the third term did not seem to justify further precision; in fact, with a divisor as large as 20 this term would be negligible in almost all cases, whereas even with the divisor of 10 its effect upon the medium or large porosity factors was very small, emphasizing the relatively small absolute contribution of these pores to percolation. It is interesting to note in figure 4 that the trend of points suggests that a percolation rate of more than about 0.1 inch per hour would be very uncommon if there were no pores drained by a 40-cm. tension and in figure 5 that rates greater than 0.01 inch would hardly be expected from pores finer than those draining at 100 cm. In both of these graphs it should be considered that the horizontal scale reading of -1.0 corresponds to no pores drained, because, as mentioned, 0.1 per cent represents the approximate limit of accuracy for the measurements of the coarse pores, and therefore even the most impervious sample might have 0.1 as a porosity factor.

P.F. #1, which gives the closest relation to percolation rates, appears to express approximately a pore-size effectiveness proportionate to the diameter of the pores. This could be expressed mathematically[6] as $\int_1^{100} D\,dx$ where D is the pore diameter and x the percentage moisture, and its value would be given graphically by measuring the area beneath a curve relating these variables within the diameter range corresponding to the tensions from 1 to 100 cm. of water.[7]

It is not established that the first power of the diameter is an exact expression, but deviation very far from the first power in either direction gives definitely poorer relations to percolation for the varied samples studied, and any attempt to relate the pore-size effectiveness in terms of tension to powers of the diameter approaching 2 results in a very poor relation to percolation. Thus it seems that the viscosity law relating viscous flow to the square of the diameter of capillary tubes[8] does not hold insofar as soil pore size based on tensions is concerned. This adds interest to the relation between percolation rate and the diameter to the 1.5 power indicated for sand separates by Nelson and Baver (22). It could be interpreted as suggestive of a gradation of some sort, sand separates being more like the capillary tubes, and aggregated silts and clays less like tubes.

As already mentioned, a number of percolation rates were measured by running water through the samples from below, with no membrane except a single layer of muslin which had scarcely any influence on the water movement even for very rapid rates of flow. This method has the advantage that it allows close observation of the reactions and behavior of each individual sample. This has

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[6] Any definite integral $\int_a^b \phi x\,dx$ can be interpreted as the area under the curve with equation $y = \phi x$ between the points b and a.

[7] 1 cm. is given here instead of 0, because a tension of 0 could not be converted into a finite diameter.

[8] Commonly known as Poiseuille's law (7).

proved to be a great advantage because the functioning of flow channels such as root and worm holes could be readily observed and notes could be made as to whether certain portions of the samples were permitting most of the water movement.

By means of such observations, the samples studied in this way were separated into a group with readily observable flow channels, and a group in which percolation appeared to be through the natural structural and textural units only. Figure 6 shows this classification of samples in relation to percolation rates and percentage of the total porosity drained by a 40-cm. tension. Percentage of the total porosity is shown here to afford a direct comparison with results of Nelson
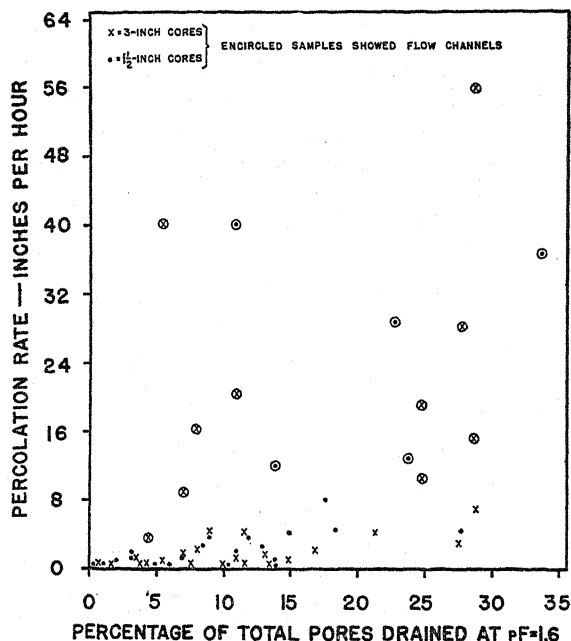

FIG. 6. RELATION BETWEEN PERCOLATION RATE AND PERCENTAGE OF THE TOTAL POROSITY DRAINED BY A 40-CM. TENSION. PERCOLATION NOT IMPEDED BY A MEMBRANE

and Baver (22). They suggested that this gave a better index to percolation than percentage porosity based on the total soil volume, but for these natural samples the result was about the same by both methods of calculation. Sands or sieved samples might be brought more in line with natural silts and clays by considering the total porosity instead of the total soil volume.

This graph (fig. 6) is kept on an arithmetic scale instead of a logarithmic scale because the main interest is in the medium and rapid percolation rates. The range in the slow rates is similar to that of figure 3. If these percolation rates are plotted against the same porosity factor as in figure 4

$$\left(\% \text{ to } 10 \text{ cm.} + \frac{\% \text{ from } 10 \text{ to } 40 \text{ cm.}}{4} \right)$$

the relation is much improved. The observational separation of samples (fig. 7) leaves a much smaller range of percolation rates within the zone designated as including samples with normal aggregation. The single sample shown as having trapped pores behaved very peculiarly, puffing up like a cake during wetting, and shrinking as it drained, releasing considerable water at a low tension. Its behavior was therefore decidedly unusual, and the results recorded involved a distinct volume change that might justify discarding them altogether.

Other samples studied by this means have fallen within the zone of trapped pores after they were kept moist in the laboratory for several weeks without toluene to prevent fungus growths. In these cases fungus growths were abundant, and it was clear that their mycelia were checking the free water flow.
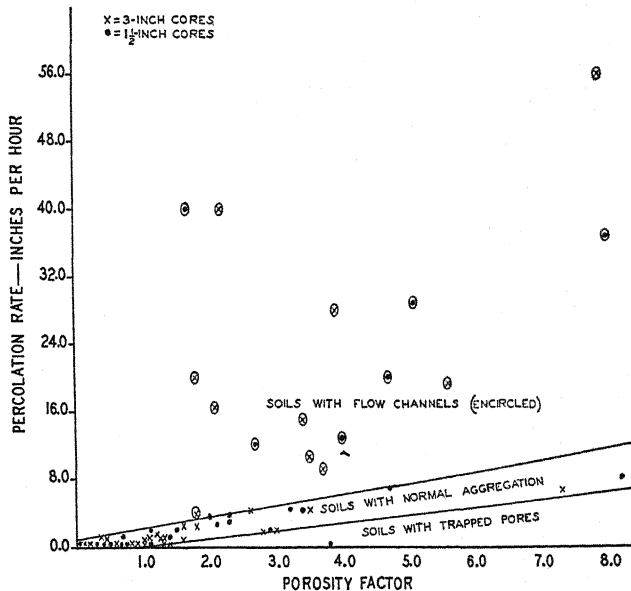

FIG. 7. CHARACTERIZATION OF SOILS BY PERCOLATION RATE AND A POROSITY FACTOR. PERCOLATION NOT IMPEDED BY A MEMBRANE

Other examples of definitely trapped pores would be expected among samples that are greatly influenced by worm or other small animal activity, when these holes become blocked.
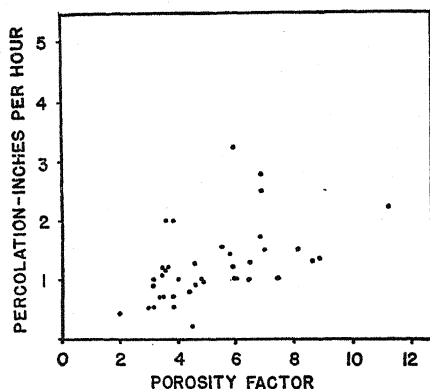
The grouping given in figure 7 helps to clarify the different conditions that are encountered, and it seems to assist in establishing somewhat of a norm for flow dominated by pores among natural structural and textural units. This norm provides a convenient reference for rating the continuity of soil pores. In fact, it is felt that the greatest practical value of percolation and moisture-tension measurements as laboratory methods lies not so much in using one to predict the other, but in using the two as a basis for characterizing the porosity of soil samples. The relationships shown in figures 4 and 5, as well as in figure 7, provide a basis for characterizing the samples, and the fact that in figure 7

the soils with normal aggregation show about the same relation between perco-
lation and the porosity factor as the samples in figure 4, where percolation was
through blotting paper, seems to support the belief that the main effect of the
blotting paper is to neutralize continuous flow channels.

At least two samples from figure 4 would fall definitely within the zone of
trapped pores if transferred to figure 7 without any correction for the effect
of the blotters, and three samples would fall in the zone of flow channels despite
the effect of the blotters. The authors feel that these designations are correct
in that they indicate a strong deviation of porosity effectiveness from that of the
majority of samples.

Cultivated samples

Only a few soil samples from cultivated areas have been studied and these
probably represent an unusual condition. They were collected in the early

Fig. 8. Laboratory Percolation through Natural, Cultivated Soil Cores Per-
forated by Earthworms in Relation to the Porosity Factor Involving Only
the Pores Drained by a 40-Cm. Tension (See Figure 4). Percolation through
a Blotting Paper Membrane

spring and were all thoroughly permeated by earthworms. Percolation rates
determined without blotting paper membranes were extraordinarily high, rang-
ing from about 10 to about 75 inches per hour. The rates were merely a reflec-
tion of the number of earthworm channels. Some cores (3-inch diameter) had
as many as 12 such worm holes through which freely flowing water could be
observed.

When percolation rates were determined through these same samples using
blotting paper membranes the results were entirely different. These values
are plotted (fig. 8) against the porosity factor already discussed, not including
the third term: P.F. = % pores to 10 cm. $+ \dfrac{\text{\% pores from 10 to 40 cm.}}{4}$. The
relation is not so close as for the sod profile samples, but it is better than the
simple relation to the percentage of pores drained at 40 cm. When the actual
values are compared with those in figure 7 it is evident that most of these samples

would fall within the zone designated as having trapped pores.   If, on the other hand, the percolation rates determined without membranes were plotted, all of these samples would occur in the zone of flow channels, which is obviously a correct designation insofar as it goes.

It is uncertain just how persistent earthworm channels are in the field or how their effect is to be evaluated, but the percolation and tension measurements in the laboratory seem suitable for their study.   If many of these channels are present there seems to be no chance for a close correlation between free-flowing percolation and any porosity factor.   When a membrane is used there is a definite relation, however, and the designation of trapped pores by figure 7 is undoubtedly correct in that the channels when blocked by the membrane are less effective than equal volumes of coarse pores among natural aggregates.   It would seem from the experience cited by Horton (13) that the free-flowing rate through earthworm channels would be only temporary, and that when, as he says, "their perforations become filled with dust," after a prolonged dry period, the infiltration capacity may be abnormally low.   This condition would seem to be more comparable to the flow through the blotting paper membrane in the laboratory, where, as indicated, the rates are abnormally low by the standard shown in figure 7.   Thus the two designations, abnormally high for free flow and abnormally low for flow impeded by the membrane, seem not without some justification in fact.

<div align="center">DISCUSSION</div>

It is clear that percolation rates determined through a blotting paper membrane are in some cases greatly different from unimpeded flow in the laboratory. This is obviously the case when continuous channels, such as worm or root holes are involved.   In a number of tests with such samples, free-flowing percolation amounted to 25 or more inches per hour, whereas flow through the membrane was 2 inches or less   The membrane is thin and pervious and it will permit rapid water movement, but only if the entire blotting paper surface is contributing.   The effect of one or a very few continuous channels through the sample is largely neutralized.

This immediately raises the question of the usefulness of a percolation measurement which arbitrarily affords a certain impediment to natural water flow. There are grounds for this objection, but as a basis for the comparison of materials, this measurement seems to afford valuable information.   In the first place, the membrane seems to have little or no influence upon flow rates that are naturally less than about 1 inch per hour (a medium rate), and little or no influence upon rapid rates up to more than 10 inches per hour, provided the percolation capacity of the soil sample is distributed over the entire sample cross section. Further, it seems that a fundamental weakness of free-flowing percolation in the laboratory as an index to water movement in the field results from the fact that root, worm, and other channels in the field usually function freely for only a limited distance, being blocked sooner or later by natural structural or textural units of soil.   On this basis it appears not unlikely that percolation rates in the

laboratory with a porous membrane impeding the water flow, may prove to be better guides to field behavior than simple unimpeded flow. The order of magnitude of the values obtained suggests this. Free flow in the laboratory can easily amount to 25 or even 50 inches per hour when channels are present, whereas infiltration in the field seldom amounts to much more than one tenth that much (10, 11, 19, 21). The practical maximum of around 10 inches per hour through blotting paper is more in line with field results.

The dominance of the coarse pores in accounting for water percolation through soils is clearly evident from this study, and the use of the volume of pores to pF = 1.6 as a single index to soil permeability as suggested by Nelson and Baver (22) seems appropriate for many purposes. The relation of this value to laboratory percolation is good compared to most other single indexes, and as a routine measurement with some apparatus such as that of Leamer and Shaw (15) the difference between many soil conditions can be clearly shown. The fact that with slow percolation rates the added factor involving percentage porosity from 40 to 100 cm. gives a somewhat closer relation to percolation, does not detract from the usefulness of the single measurement at pF = 1.6, nor does the heavier weight attached to the coarsest pores in accounting for the more rapid rates. These measurements represent refinements that will prove useful under certain circumstances, and they help to clarify the general relation between percolation rate and the effective pore sizes of silt and clay soils.

In this study, no special effort was made to remove all the air because the idea was to obtain results applicable to field conditions insofar as possible. It now appears that the influence of the air that was trapped by the techniques employed is of considerable importance in explaining these results as well as in explaining similar behavior in the field. Indications have been reported (27) that trapped air tends to be concentrated in the tortuous intermediate soil pores, where it is neither expelled by sorption and tension nor flushed out by momentum. The contribution of the intermediate pores would, therefore, be largely a result of direct pore channels which would give rise to relatively rapid water movement. On the other hand, since most of the large pores were filled with water, their contribution would be the result of both direct and tortuous pore channels. They would, therefore, be less effective per unit relative to their diameter.

Trapped air may not be the only factor involved in causing percolation to be related more closely to the diameter of the pores than to the diameter squared, but it appears to be one important factor and it would seem adequate to account for many of the results obtained. This applies, of course, only to comparisons of pores that are similar in nature. The deviations from the normal or average relation between percolation and pore size, as already discussed, undoubtedly reflect real differences in pore continuity. A closer relation to any power of the effective pore diameter is to be expected only by limiting the comparisons to samples the pore spaces of which are more uniform in character.

Limited experience in studying soils wetted in a vacuum suggests that as a laboratory procedure, the removal of air may be desirable because it enables

closer duplication of results and prevents to a large extent the disruption of the physical state of the soil sample. The elimination of these difficulties is a great advantage in many cases, although the resulting interpretation of comparisons with field behavior becomes correspondingly less direct. Some of the differences in capillary behavior introduced by the removal of air have been shown by Oehler (23).

From these studies, one other suggestion seems worthy of note. It concerns the sealing of soil surfaces in the field, which has been shown to exert a great influence upon infiltration rates (9, 10). This phenomenon is difficult to duplicate in the laboratory, and from consideration of Darcy's law, it seems unlikely that a surface seal would account for extremely slow percolation rates because the thickness of the sealed layer is so small compared to the entire length of any soil column. But the operation of Darcy's law requires the presence of a continuous column of water and does not take into account the influence of trapped air. It would seem, therefore, that the influence of a surface seal is more a case of sealing the air in than of sealing the water out. Some indications of the air pressure built up and the general behavior of trapped air in sand columns have been given by Free and Palmer (12). This type of behavior in the field would account for many slow percolation rates and would play a particular role in the surface seal. A very thin layer of dense soil would, by the surface tension of the water, prevent the escape of air from coarser pores beneath this dense layer. This would prevent the entrance of water or the establishment of water columns which could function in accordance with Darcy's law. This appears to explain laboratory behavior and it would be expected to operate equally well in the field. A very thin, dense soil layer seems to be essentially impervious in the laboratory only when it is in contact with air.

SUMMARY

Simple laboratory procedures for the determination of percolation rates of undisturbed soil samples are described. The rate of percolation of normally aggregated soils was not affected appreciably by direction of percolation or by the use of muslin or blotting paper membranes. Soils having worm holes or other continuous large flow channels showed extremely high rates of percolation when only a muslin membrane was used. In such cases the presence and behavior of these flow channels could be readily observed. The use of blotting paper membranes gave results with these samples that are believed to be more like field percolation rates.

The effective pore-size distribution was measured by determining water removal at tensions of 10, 40, and 100 cm. of water, and the relationship to percolation rates was studied. The porosity factor used by Nelson and Baver (22) gave fairly satisfactory results, but a more accurate picture of the relationship was obtained by using a porosity factor as follows: % pores drained at 10 cm.

$$+ \frac{\% \text{ pores drained between 10 and 40 cm.}}{4}$$

$$+ \frac{\% \text{ pores drained between 40 and 100 cm.}}{10}$$

Pores drained at tensions from 40 to 100 cm. make only very small absolute contributions to percolation and may be omitted from the factor except for soils having very slow rates.

These results indicate that the water-filled pores contribute to percolation approximately in proportion to their diameters rather than to the square of their diameters as required by the law of viscous flow. Reasons for this appear to be associated with the trapping of soil air.

Neither percolation rate nor pore-size distribution measurement alone is sufficient to characterize water movement in soils. The two determinations should be used to supplement each other in affording a picture of the momentary physical condition of a particular soil with respect to water movement.

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HYDRATION CONTROL OF MONTMORILLONITE AS REQUIRED FOR ITS IDENTIFICATION AND ESTIMATION BY X-RAY DIFFRACTION METHODS[1]

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INTRODUCTION AND LITERATURE REVIEW

Montmorillonite[3] occurs rather generally in soils, but its occurrence there has been overlooked in many cases, or the amount present underestimated, because of inadequate control of conditions affecting its distinguishing crystal properties in x-ray diffraction analysis. It has been shown (1, 6, 11) that degree and uniformity of hydration of montmorillonite markedly influence the character of the x-ray diffraction pattern produced. When all of the crystal plates are uniformly hydrated, for instance with two or three molecular layers of water within the interplate space, then a strong diffraction line will appear at a spacing somewhere between 14 and 18 A. If some of the crystal plates are insufficiently hydrated, however, montmorillonite gives a diffraction line at 10 or 12 A. spacing (characteristic of hydrous mica), and a correspondingly weaker diffraction line at 14 to 18 A. spacing, which is the line critical for identification (11) and estimation (6) of montmorillonite.

For the estimation of montmorillonite in a soil clay containing a mixture of clay minerals, the objective must be, first, to prevent montmorillonite from producing a 10 A. diffraction line which would be confused with a similarly spaced line from hydrous mica, and second, to maximize the intensity of the (001) montmorillonite line at a spacing between 14 and 18 A. If this objective is attained then any 10 or 12 A. diffraction line found in the pattern can be correctly attributed to the hydrous mica or mica-like fraction. Moreover, the intensity of the (001) montmorillonite line, being then at a definite maximum, can be used to estimate quantitatively the amount of montmorillonite present in various samples, by visual or photometric comparison with intensities produced by synthetic mixtures of pure minerals (6, 7).

It seemed reasonable to postulate that the aforementioned objective might be attained by adequate control of the degree of hydration of montmorillonite; however, the wide variety of preparatory procedures in use for x-ray diffraction analysis of clays (2, 3, 4, 5, 8, 13, 16) attests to the differences of opinion as to what constitutes adequate hydration control. Studies in this laboratory (1, 6, 7, 11) have shown that the degree and the uniformity of hydration of montmorillonite are greatly influenced by three more or less independent factors; namely, (a) the nature of base saturation of the clay, (b) the physical condition of the sample powder engendered by the method of preparation and drying, and (c) the moisture content of the atmosphere with which the sample is allowed to come into equilibration prior to and during x-raying. The methods for x-ray diffraction analysis of clays described in the literature vary widely in their attention to these three factors: some methods pay little or no attention to them, whereas others recognize the importance of only one or two of them.

Kelley, Dore, and Page (13) saturated three clays from alkali soils with calcium, and then obtained distinct 15 A. diffraction lines, in contrast to results with clays from other alkali soils which were allowed to remain with the original cation saturation. Kelley *et al.* (12) employed calcium saturation of clays representative of California soils and found weak, medium, or strong 15 A. lines from some of the clays, but not in several of the others. In their studies, the relative humidity was adjusted to 30 per cent (over 52 per cent H_2SO_4 by volume), which is rather too low for optimal hydration of montmorillonite.

Nagelschmidt (15), working with 50 per cent relative humidity, noted an increase in the (001) montmorillonite spacing from 12 A. with potassium saturation to about 15 A. with calcium saturation. No advantage, however, appears to have been taken of the benefit of calcium saturation, since the procedure as outlined for the aggregate x-ray diffraction method (16) indicates that the clays were allowed to remain in their original cation saturation; humidity control is not specified.   Similarly, Clark, Grim, and Bradley (3) apparently did not change the original cation saturation in their procedure for x-ray diffraction analysis of clays and did not control the relative humidity.   Favejee (4) adopted a standard humidification at 44 per cent (over saturated $KHCO_3$) but did not change the original cation-saturation.

Maegdefrau and Hofmann (14) recognized the importance of relative humidity as influencing the (001) line of montmorillonite. They recognized the fact that montmorillonite gave an (002) line at a spacing of about 10 A. with humidities near 98 to 100 per cent, and also showed the importance of using a rather high relative humidity for avoidance of spacings of less than 14 to 15 A. Their work was conducted with montmorillonite from bentonites. Montmorillonite from certain soils has been found (1, 6, 11) to be even more subject to decreased spacing when exposed to a relative humidity of less than 50 per cent.

Gieseking (5) saturated clays with large organic bases in order to intensify the 14 to 18A. line of montmorillonite. With clays given such treatment, the humidity conditions appeared not to affect the (001) spacing greatly, at least for montmorillonite from certain bentonites. It has been shown (11), however, that for certain soil clays and argillaceous deposits, the saturation with large organic cations does not bring out the 14 to 18 A. line, whereas under the same (suitable) conditions of humidity, saturation with calcium did.

The procedures of Hendricks *et. al.* (2, 8) also employ the original base saturation, and do not specify the relative humidity. Little or no montmorillonite was identified in the samples they examined. However, Hendricks, Nelson, and Alexander (9) have shown that the characteristic montmorillonite diffraction line is obtained over a wider range of relative humidity when calcium is used for cation-saturation than when sodium, potassium, hydrogen, or certain other cations are used. Hendricks, in a personal conversation with the authors[4], said that he believed the use of calcium saturation would be desirable in laboratories where the relative humidity was low.

In all the procedures enumerated above, the sample was dried from water or alcohol,

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[4] November, 1941.

and in consequence it was in a firm physical condition, difficult to hydrate. The relative humidity, when controlled at all, was usually held at from 30 to 50 per cent, whereas the optimal level (6) is in the neighborhood of 90 to 92 per cent. The procedure recently described by Jackson and Hellman (11) provides for saturation of the clay with calcium and drying of the clay from benzene. Calcium saturation of the clay results in relatively high and uniform hydration of the clay through the rather wide range of relative humidities commonly occurring in the laboratory. At the same time, the clay dried from benzene is an incoherent powder, which experience has shown equilibrates rapidly with atmospheric humidities to which samples may be exposed. This facility of hydration stands in sharp contrast to the difficulty of uniform hydration experienced with hard films and oriented film aggregates obtained by methods involving drying the clay from water or alcohol; the facility of uniform hydration was judged by the intensity of the 14 to 18 A. diffraction line and the absence of a 10 A. line obtained with pure montmorillonite. When x-rayed, a variety of clays thus saturated with calcium and dried from benzene showed an intensified diffraction line for montmorillonite at about 15 A. spacing, and indicated the presence of more or less montmorillonite in soil clays generally. Some soil clays which earlier had been characterized as being principally hydrous mica were shown under these conditions of preparation to consist largely of montmorillonite (11).

It has been observed more recently that exposure of clay samples thus prepared to the laboratory atmosphere of varying relative humidities resulted in a variation in the spacing and intensity of the (001) montmorillonite line (1, 6). In addition, a weak 10 A. montmorillonite line, either (001) or (002) depending on whether the humidity of the laboratory atmosphere was very low or very high, sometimes occurred in association with the (001) line at about 15 A. spacing (1, 14). Tests involving controlled humidities of various levels showed that an intense (001) line at 16 A. spacing was produced consistently by exposure of montmorillonite to air of 92 ± 1 per cent relative humidity for 12 to 15 hours prior to and during x-raying (6). This close control of the humidity, though easy to obtain in a static system such as that prevailing in a desiccator containing an appropriate solution, is more difficult to achieve in a dynamic system where air is moving through the x-ray diffraction camera. To overcome this difficulty, an air humidifier suitable for close control of the humidity of moving air was developed and is described later in this paper.

Despite apparent equilibration of the sample with air of 92 ± 1 per cent relative humidity, it was found that similarly prepared samples of montmorillonite sometimes showed a variation in the intensity of the 16 A. diffraction line; occasional samples also showed a weak 10 A. line (6). These results indicated that the control of hydration of montmorillonite was still somewhat uncertain. Further study showed that more careful control of the hydration of montmorillonite prior to drying from benzene was necessary to ensure consistent diffraction patterns. In this paper, the experiments involved in the development of a suitable procedure for clay hydration control in benzene suspension are described first, after which the details of the entire preparatory procedure finally adopted are given.

CONTROLLED HYDRATION OF MONTMORILLONITE PRIOR TO DRYING

In preparing clays for x-ray diffraction analysis according to the procedure of Jackson and Hellman (11), it is necessary to change the suspension medium of

the clay from water to benzene after completion of base-exchange saturation of the clay with calcium. Since water and benzene are immiscible, this change cannot be made until the water held by the clay has been replaced largely by a medium which is miscible with benzene and which can be removed from the clay with subsequent benzene washings. The water on the clay is therefore replaced by two 50-ml. washings with 70 per cent methanol followed by three 50-ml. washings with absolute methanol. Three 30-ml. washings with benzene suffice to replace the methanol. The clay is dried at this point. The transition from a water-wet clay to a benzene-wet clay is thus the last step in the procedure which can definitely affect the hydration level of the clay prior to drying. Depending upon how completely the methanol washings have replaced the water, varying quantities of water are left on the clay. The modifications in the procedure of Jackson and Hellman (11) proposed and described herewith provide for the controlled addition of minute but definite quantities of finely dispersed water to the clay suspended in benzene just prior to drying.

The montmorillonite used in the experiments to be described consisted of fine clay separated from a Wyoming bentonite from Upton, Wyoming[5] (W1021). This fine clay fraction (particles $< 0.2\ \mu$ in diameter) was separated by means of a centrifuge procedure essentially as described by Truog et al. (18, 19) except that the hydrogen peroxide treatment and the sulfide treatment for iron removal were omitted. The fine clay was conditioned, Ca-saturated, and washed with methanol and benzene according to the procedure of Jackson and Hellman (11, p. 142), except that in certain tests ethyl alcohol of various concentrations was substituted for 99 per cent methanol just prior to the benzene washings. A stock suspension of the undried clay in methanol was made, and aliquots were then taken for the various tests. This same technique was employed in preparing stock suspensions of hydrous mica, other montmorillonites, and the soil fine clays used in this investigation.

Introduction of hydration water

In a preliminary investigation, it was observed that the use as a washing medium for transition from water to benzene of a solution of 80 per cent ethanol and 20 per cent water by volume resulted occasionally in a clay preparation which dried to a fluffy, incoherent powder in contrast to the soft aggregates often obtained when absolute methanol was used as the transition medium. Upon x-raying, the incoherent powder gave a diffraction pattern showing a fairly intense 16 A. (001) line and little or no 10 A. line. This observation corroborated a result previously obtained (11) when a desert soil fine clay wetted with CS_2 was dried; the CS_2 evaporated so rapidly as to cause considerable cooling and condensation of water on the clay, and the sample gave a greater intensity of the 16 A. (001) diffraction line than occurred after any other mode of preparation. These results suggested that small quantities of water should be added to the clay suspended in benzene just prior to drying.

Direct addition of this water to the benzene suspension of clay was impracticable because of the small quantity of water needed, and the impossibility of disseminating it throughout the suspension so that it would be taken up uniformly by the clay.   Tests were made of the possibility of hydrating the clay by washing it repeatedly with a ternary solution consisting of water in true solution in benzene and ethanol.   The solution in each test was saturated with respect to water.   Clay washed with such ternary solutions in which only a small amount of water and ethanol were dissolved dried as the soft aggregates, indicating insufficient hydration.   Clay washed with ternary solutions in which more water and, of necessity, more ethanol were dissolved, became solvated with both water and ethanol and dried to hard films, the beneficial effects of drying from nonpolar benzene being lost.   It thus appeared that to obtain the incoherent clay powder characteristic of properly hydrated clay, the water must be sorbed as a separate phase by the clay while suspended in benzene.   Such a condition could be obtained by expulsion of a water phase from an ethanol-water solution added to benezene in which the clay was suspended.

Accordingly, tests were made in which a series of solutions consisting of various percentages of water in ethanol were added to a series of clay suspensions each consisting of 50 mgm. of montmorillonite fine clay in 50 ml. benzene.   It was found that the samples dried to the incoherent, powdery physical condition which is associated with the intense 16 A. (001) diffraction line of montmorillonite only when 1-ml. portions of ethanol-water solutions containing 7 to 8 per cent by volume of water had been added.   These results were corroborated by the addition of varying amounts of an ethanol solution containing 8 per cent water by volume to a second series of suspensions.   Only those suspensions receiving 0.9 to 1.1 ml. of the ethanol solution produced a powdery condition of the dried clay.

Sometimes the dried sample thus prepared was found to contain hard pellets, whereas the bulk of the clay remained in the incoherent powdery condition suitable for x-ray diffraction analysis.   The formation of pellets was attributed to an excessive wetting of a part of the sample which collected in the droplets of water formed at the interface between the added ethanol-water solution and the benzene suspension.   Upon drying, this excessively wetted clay formed hard pellets.   To avoid this difficulty, a method was developed for introducing the required water into the clay by means of a ternary solution of benzene, ethanol, and water, which was near saturation with respect to water.   The clay was thoroughly dispersed in this solution and then benzene was added so as to lower its solvent power for water and thus set free from solution just enough finely disseminated water to hydrate the clay properly.   This method gave the clay a uniformly satisfactory physical condition and hydration.

Measurement of hydration water transferred to clay from ternary solution

A satisfactory method for *uniformly* hydrating the clay while suspended in a ternary solution consisting largely of benzene with small amounts of ethanol and water thus being at hand, there remained the problem of determining the

quantity of water required to hydrate the clay properly. It is possible to measure the quantity of water transferred to the clay by determining the difference between the original quantity of water in the ternary solution and the amount remaining therein at the conclusion of the hydration process.

The amount of water present in solution at the saturation point in the ternary suspension medium is given for a series of possible ethanol and benzene contents by the curves in figure 1. For benzene-ethanol ratios up to 25:1, the curves are based on the first appearance of the cloudy point when benzene is added to ethanol-water solutions; and for benzene-ethanol ratios richer in benzene than 25:1, by the appearance of a cloudy point when water is added to a benzene-ethanol solution. The change in procedure above the 25:1 ratio is necessary because the water capacity of the system begins to increase rather than decrease with the addition of benzene at benzene-ethanol ratios much higher than 25:1. These data were calculated over to the basis of quantities of water and benzene
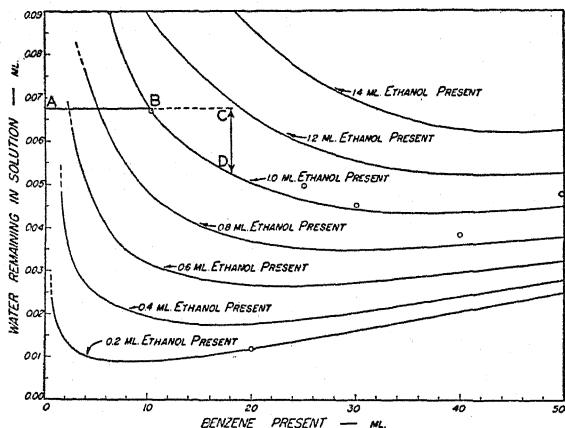

FIG. 1. SOLUBILITY OF WATER IN BENZENE-ETHANOL SOLUTION AS THE PROPORTIONS OF BENZENE AND ETHANOL VARY

held in solution by 1 ml. of ethanol, and the points thus obtained were plotted on the curve labeled "1.0 ml. ethanol present" (fig. 1). The curve for "0.2 ml. ethanol present" was derived by simple proportion from the 1.0 ml. ethanol curve, since all quantities involved would be one fifth as great. The other curves in figure 1 were established similarly. One datum point was placed on the 0.2 ml. curve since it established the increased solubility of water in systems with high benzene-ethanol ratios, but if plotted on the 1 ml. curve its position would be beyond the graph. These curves were checked with data calculated from Seidell (17, p. 1093) and the International Critical Tables (10, p. 404) and were found in general agreement. Sufficient variation existed, however, between these curves and those based on data given in the literature as well as those derived by using different sources of benzene, to make it advisable to characterize each lot of benzene before use.

As an illustration of the use of the curves in figure 1, line *CD* represents the

amount of water expelled from a ternary system containing 1.0 ml. of ethanol
and 0.0675 ml. of water upon the addition of 18 ml. of benzene.   Before addition
of the benzene, the 1.0 ml. of ethanol contains 0.0675 ml. of water as represented
by point A; as benzene is added, all of the water remains in solution to point B
(11 ml. of benzene present).   As more benzene is added, water is expelled, and
the composition of the ternary suspension medium follows along the curve from
B to D.   At the point D (18 ml. of benzene present), 0.0530 ml. of water remains
dissolved, as compared to the initial 0.0675 ml. dissolved.   The difference,
represented by line CD (0.0145 ml.), is the water expelled from solution as a
second phase which becomes sorbed on the clay.   Expulsion of water in this
manner can be controlled to within 0.001 ml. by control of benzene addition to
within 0.1 ml.

TABLE 1

*Influence of degree of hydration of montmorillonite on its physical condition after drying**

AMOUNTS OF THREE LIQUIDS CONTAINED IN THE SUSPENSION MEDIUM CONTAINING 0.052 GM. OVEN-DRY MONTMORILLONITE			WATER TRANSFERRED TO MONTMORILLONITE ON ADDING BENZENE		PHYSICAL CONDITION OF DRIED MONTMORILLONITE SAMPLES
Water	Ethanol	Benzene	Per sample	Percentage of oven-dry sample	
ml.	*ml.*	*ml.*	*ml.*		
.10	.90	8	0.038	73	Hard films
.09	.91	9	0.034	65	Hard films
.08	.92	11	0.028	54	Soft, incoherent powder
.08	.93	25	0.023	44	Soft, incoherent powder
.06	.94	31	0.016	31	Soft aggregates
.05	.95	53	0.008	15	Soft aggregates
.04	.96	53	0.000	0	Soft aggregates

* The montmorillonite was suspended in a benzene-ethanol-water solution, hydrated by
adding benzene so as to provide free water, and then dried after decantation of the super-
natant liquid.

Quantity of water required for proper hydration of montmorillonite

To determine the quantity of water needed for the proper degree of hydration
of montmorillonite, varying amounts of water were transferred to a series of
0.052-gm. samples of benzene-wetted clay in accordance with the procedure just
described.   The proportions of the liquids were adjusted in accordance with the
curves in figure 1 so as to vary the quantities of water set free for sorption by the
clay.   These quantities and the physical condition of the corresponding samples
thus prepared are reported in table 1.   It was found that only those samples
hydrated to 44 and 54 per cent of their oven-dry weights dried to a satisfactory
physical condition.   These samples gave x-ray diffraction patterns in which the
lines were sharply defined and the intensity of the 16 A. (001) line for mont-
morillonite was maximized (fig. 2, I).   The samples with a lower degree of hydra-
tion resembled preparations dried from anhydrous benzene, whereas those with
excessive hydration resembled preparations dried from water.   Similarly, the

x-ray diffraction patterns of samples involving low hydration resembled those given by preparations dried from anhydrous benzene (fig. 2, II), and patterns of samples involving excessive hydration resembled those given by preparations dried from water (fig. 2, III).   Thus, with either excessive or deficient hydration of the montmorillonite, a 10 A. line appeared, and a loss in intensity of the 16 A. line occurred.

In further tests, the benzene-ethanol ratio of the hydrating medium was kept constant (as was later shown to be necessary), and the quantity of water was varied.   Results with montmorillonite fine clay separated from various bentonites and with montmorillonite bearing fine clays from the soils Miami B_2 (Wis.) and Spencer B_2 (Wis.) and, for comparison, with a hydrous mica clay sample (W1016, from the Goose Lake area) led to the conclusion that hydration
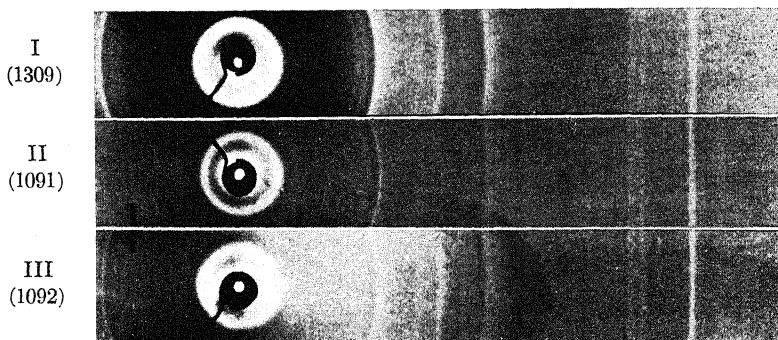

FIG. 2. INFLUENCE OF PREPARATORY PROCEDURE ON X-RAY DIFFRACTION PATTERNS OF MONTMORILLONITE

The samples were taken from the same Ca-saturated stock, and x-rayed at 92 per cent relative humidity.

I. Hydrated and dried according to controlled hydration procedure adopted.

II. Dried from anhydrous benzene at 40 per cent relative humidity (inadequately hydrated).

III. Dried from water (inadequately hydrated).

to the extent of 50 per cent of the dry weight of clay was optimal.   The clay thus hydrated dries to an incoherent powder, somewhat softer than that dried from benzene alone.   The advantages of drying from a liquid of low polarity are thus retained, and there is gained the improvement resulting from the addition of small quantities of water.   Experience has shown that a sample in the incoherent physical condition readily reaches equilibrium with any desired humidity, and it is this result possibly as much as the actual hydration prior to drying which accounts for the marked advantage of the ternary solution procedure.

Permissible ethanol content of ternary suspension medium

A certain quantity of ethanol remains dissolved in the water set free from the ternary solution in which the clay is suspended during the hydration procedure. Small changes in the ethanol content of the suspension medium result in consid-

erable changes in the ethanol content of this water-ethanol phase which is set free and sorbed by the clay. Since preliminary tests had shown that clay samples which had sorbed too much ethanol dried to a poor physical condition, a series of tests were made to determine the maximum permissible ethanol content of the ternary solution.

To each of several samples of montmorillonite suspended in the ternary solution containing varying amounts of ethanol, identical weights of water were transferred by adding appropriate quantities of benzene. The hydrated samples were allowed to dry and then were x-rayed in an atmosphere of 92 per cent relative humidity. It was found with a final ethanol content of 4 per cent by volume at the conclusion of the hydration process that the 16 A. diffraction line was at a maximum and the 10 A. diffraction line (coincident with that of mica) was absent. As the final ethanol content of the ternary solution was increased stepwise from 4 to 16 per cent, the corresponding montmorillonite samples dried to increasingly harder aggregates which on x-raying gave 16 A. (001) lines of decreasing intensities in association with 10 A. (001) lines of increasing intensity. In order to avoid a possible misinterpretation as regards the presence and content of mica in clay samples on the basis of the appearance and intensity of the 10 A. (001) line, it was concluded that the final ethanol content of the clay ternary suspension medium on completion of the hydration process should not exceed 4 per cent by volume. In the procedure adopted, a final benzene-ethanol ratio of 30:1 was chosen, giving a concentration of 3.3 per cent ethanol. Final ratios of benzene to ethanol as high as 50:1 (2 per cent ethanol) or even higher have been found to be satisfactory; however, water transfer, begun at the 11:1 ratio, continues on addition of benzene to the ternary solution only to a ratio of about 30:1, and then ceases, as shown in figure 1 by the slight rise in the curves to the right of 30:1 benzene-ethanol ratios.

Removal of supernatant liquid from the clay suspension

The hydration level, now carefully established, must be maintained during removal of the benzene by drying of the clay. It is evident that the more rapid evaporation of benzene in comparison with that of the other constituents of the tenary solution during the drying of the hydrated clay is equivalent to a reversal of the benzene addition (fig. 1) and causes an increase in the solubility of water in the remaining ternary solution. This evaporation therefore results in the passage of water from the clay back to the remaining solution. In order to prevent this partial dehydration of the sample during drying, as much as possible of the supernatant liquid is decanted from the flocculated clay prior to drying, thereby decreasing to within safe limits, the quantity of water which might thus be removed from the clay. The moisture content of a number of clay samples dried after decantation of the supernatant liquid was found to be somewhat higher than that of similar samples dried without this decantation (table 2). This fact is of importance, since any procedure which allows a reduction in the moisture content of the sample once it has become properly hydrated works at cross purposes to the main objective. As an additional advantage, decantation

of the supernatant liquid reduces the drying time from 3 hours to approximately 5 minutes.

PROCEDURE FOR CONTROLLED HYDRATION OF CLAY SAMPLES PRIOR TO AND DURING X-RAYING

Details of the procedure for controlled hydration of clay prior to drying, as adopted on the basis of the tests just described, follow, together with a description of methods and apparatus for controlled humidification of the clay powder prior to and during x-raying.

TABLE 2

Effect of decantation of the supernatant ternary suspending and hydrating solution from clays on their moisture content after drying in a tray at 30°C. and 65 per cent relative humidity for 24 hours

The moisture content was determined by weighing before and after drying at 110°C.

SOURCE OF FINE CLAY	DISPOSAL OF SUPERNATANT LIQUID PRIOR TO DRYING	WEIGHT OF SAMPLE USED FOR MOISTURE DETERMINATION (110°C.)	WATER LOSS BETWEEN 30 AND 110°C.	
		gm.	gm./sample	per cent
Spencer (Colby) silt loam, B_2 (Central Wis.)	Decanted	.0347	0.0079	22.7
	Not decanted	.0337	0.0049	14.5
Miami silt loam, B_2 (Southern Wis.)	Decanted	.0278	0.0032	11.5
	Not decanted	.0385	0.0041	10.6
Parr (Carrington) silt loam, B_2 (Southern Wis.)	Decanted	.0043	0.0055	11.6
	Not decanted	.0086	0.0008	9.3
Bentonite (W1010) (Eastern Wyo.)	Decanted	.0260	0.0064	24.6
	Not decanted	.0347	0.0083	23.9
Bentonite (W1021) (Upton, Wyo.)	Decanted	.0217	0.0025	11.5
	Not decanted	.0232	0.0020	8.6
Hydrous mica (W1016)* (Goose Lake Area, Ill.)	Decanted	.0195	0.0018	9.2
	Not decanted	.0241	0.0017	7.0

* Clay kindly supplied by R. E. Grim, Illinois State Geological Survey, Urbana, Ill.

Hydration of suspended clay in ternary solution

Fine clay is conditioned according to the procedure of Jackson and Hellman (11, p. 142) up to and including base-exchange saturation with calcium, and washing with methanol to remove soluble salts. An aliquot of about 0.3 gm. of clay is then washed three additional times with 50-ml. portions of absolute methanol to free it rather completely of water. Sufficient methanol is next added to this sample to form a suspension of convenient concentration for pipetting 50-mgm. aliquots of clay for diffraction analysis. This 50-mgm. subsample, usually taken with a Mohr pipette, is placed in a centrifuge tube, and washed once again, with 30 ml. of absolute methanol to provide a sample as free

as possible of water.   The sample is next washed three times with 30-ml. portions of benzene to free it of methanol.

Addition of ternary solution.   To the 50-mgm. benzene-wetted sample remaining in the bottom of the tube and from which the supernatant benzene has been decanted, there is now added 10 ml. of a ternary solution made up as follows: Exactly 20 ml. of absolute ethanol and 1.35 ml. of water are added to a 200-ml. volumetric flask.   Benzene is then added to give a volume of approximately 195 ml. and the mixture shaken and warmed to 25°C., after which benzene is added to bring the volume to 200 ml.

Addition of benzene.   The centrifuge tube containing the clay and 10 ml. of ternary solution is stoppered and vigorously shaken so as to disperse the clay thoroughly in the solution, after which the stopper is removed and 21 ml. of benzene is added to the tube from a burette.   The tube is again stoppered and shaken vigorously.   The addition of this amount of benzene sets free an amount of water from the ternary solution equal to approximately 50 per cent of the dry weight of the clay, and gives a ratio of benzene to ethanol in the tube of approximately 30 to 1 (relationships based on figure 1).   If samples smaller than 50 mgm. are taken, proportionately less ternary solution and benzene are used (ml. of ternary solution needed = 0.2 × sample weight in mgm.; ml. benzene needed = 2.1 × ml. ternary solution used).

Drying the sample.   The clay is next allowed to settle in the tube, after which most of the supernatant liquid is decanted.   The clay and remaining liquid are now poured into the center of an 8- by 10- inch photographic developing tray, the inside bottom of which is slightly convex so that the supernatant liquid drains to the edges of the tray and may be decanted, leaving the clay deposited in the form of large floccules in the central area of the tray.   The tray is immediately placed in a chamber regulated at 65 per cent relative humidity and 30°C., and the clay allowed to dry.   Drying the clay in this manner prevents condensation of moisture on the tray from the atmosphere caused by the rapid cooling of the tray on evaporation of the benzene, and at the same time maintains the relative humidity at a sufficiently high level to prevent excessive dehydration of the sample during drying.   This procedure was found to be the most effective for obtaining the incoherent powdery condition of the sample needed to assure uniform hydration of the individual crystal lattice plates during subsequent humidification at any chosen air humidity.   Drying at 30°C. occurs within 5 minutes, after which the clay is immediately brushed from the tray into a small vial, which is then tightly stoppered.   For maximum intensity of the montmorillonite pattern, not more than 24 hours should elapse from the time of drying the clay sample to the time when it is subjected to humidification.

Humidification of clay powder for x-raying

Preliminary humidification.   A weighed portion of the clay sample is now pressed by means of two glass slides into the notch of the 90° wedge mount of the x-ray camera, thus giving constant volume-weight of the clay to be x-rayed, as discussed elsewhere (7).   The clay in the mount is then humidified by placing

it for 12 to 15 hours in a desiccator in which a relative humidity of 92 per cent is maintained by a saturated solution of $Na_2SO_4 \cdot 10H_2O$.

Humidification during x-raying. An atmosphere of 92 per cent relative humidity is also maintained about the sample while it is being x-rayed. Air of 92 per cent relative humidity, obtained from a specially designed humidifier (fig. 3), is passed through the camera at the rate of 6 liters per minute. The circular diffraction camera is enclosed with brass side plates, each provided with
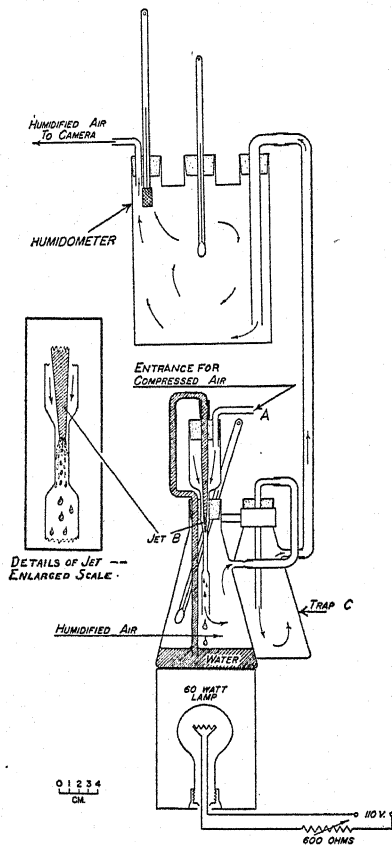

FIG. 3. AIR HUMIDIFIER

openings so located at diagonally opposite positions as to ensure maximum circulation of air.

In the air humidifier, the stream of air enters at A (fig. 3) and becomes intimately mixed with water aspirated from the jet at B. The humidified air then passes to the trap C, which catches any water in the form of a mist, and is thence led through the humidometer and to the x-ray diffraction camera. The sweep of air past the jet B causes water to circulate continuously from the supply in the bottom of the flask through the jet. Here the water is broken up into a fine mist and intimately mixed with the air, causing full saturation of the air.

Maintenance of room temperature throughout the humidifying unit would result in nearly 100 per cent relative humidity. Both water and air, however, are cooled as much as 5°C. by the vaporization of water and the adiabatic expansion of air on leaving the capillary below jet *B*. This temperature drop results in the air being saturated with water at a lower temperature. Subsequent warming of the air to room temperature, in passing through the humidometer and the x-ray diffraction camera, causes a drop in the relative humidity to as low as 75 per cent. Control of the temperature of the water in the humidifier flask, however, permits the selection of a suitable temperature differential between the humidifier and the x-ray diffraction camera. The temperature differential is satisfactorily estimated by the difference in temperature registered by the thermometer inserted in the air stream in the humidifier and the dry bulb thermometer of the humidometer (fig. 3). It was found possible to maintain any desired relative humidity between 75 and 100 per cent with water in the humidifier. Substitution of sulfuric acid solutions of appropriate concentration in the humidifier was found to facilitate selection of lower relative humidities when desired. The temperature control is obtained by placing the humidifier on a heater constructed from a metal can and a 60-watt electric light bulb (fig. 3). A 600-ohm variable resistance placed in series with the light bulb provides suitable variation in heating.

The humidometer consists of a three-neck Woulff bottle fitted with wet bulb and dry bulb thermometers for relative humidity measurement. In preliminary trials, when a U-tube was employed to support the wet and dry bulb thermometers, the passage of the air from the constricted sidearm into the wider U-tube resulted in cooling of the air due to adiabatic expansion. This cooling resulted in occasional condensation of water on the dry bulb, thus invalidating relative humidity readings based on wet bulb and dry bulb temperatures. The provision of a tube of comparatively large diameter leading into the bottle together with a bottle of ample capacity minimizes the expansion and cooling of the air. Air of 92 ± 1 per cent relative humidity can be produced at dry-bulb temperatures ranging from 22 to 28°C. by heating the humidifier sufficiently to maintain a temperature differential of 1.0°C. between the wet and the dry bulb.

APPLICATION OF CONTROLLED HYDRATION TO X-RAY ANALYSIS OF SOIL CLAYS

The addition to the original procedure (11), of provisions needed to ensure a proper degree and uniformity of hydration of montmorillonite, has brought about marked increases in the intensity of the (001) line characteristic of montmorillonite in diffraction patterns of clays containing mixtures of montmorillonite with other clay minerals. For example, the diffraction patterns of certain fine clays high in montmorillonite from the B_2 horizons of Miami silt loam from Dane County, Wisconsin, and Parr (Carrington) silt loam from Columbia County, Wisconsin, show a marked intensification of the (001) montmorillonite line compared to those obtained when hydration was controlled only by Ca-saturation and drying from benzene (fig. 4). For comparison, the poor definition of diffraction lines resulting from drying the clay from water as opposed to benzene
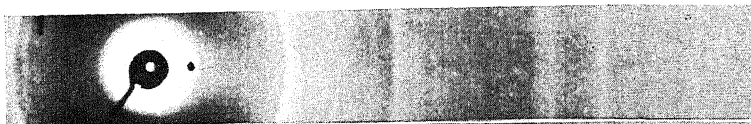
Miami silt loam, B₂ horizon. Clay high in montmorillonite; marked increase in (001) line intensity in I.

Parr (Carrington) silt loam, B₂ horizon.   Clay medium-high in montmorillonite; marked increase in (001) line intensity in I.

Hagerstown soil, B₂ horizon. Clay medium-low in montmorillonite; very marked increase in (001) line intensity in I.
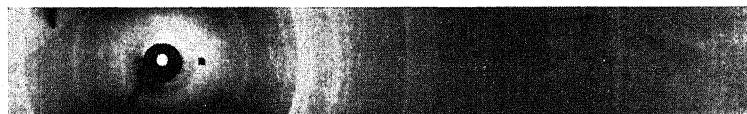
Fullerton soil, B₂ horizon.   Clay low in montmorillonite; distinct (001) montmorillonite line in I, and little or none in II.

FIG. 4. INFLUENCE OF DEGREE AND UNIFORMITY OF HYDRATION OF CLAYS ON THE RESULTANT X-RAY DIFFRACTION PATTERNS, PARTICULARLY THE (001) LINE OF MONT-MORILLONITE, INDICATED BY THE BLACK DOTS

The samples were fine clays from the soils indicated, and were calcium-saturated prior to drying. The change in type of beam stop from I to II is inconsequential. In prepara-

is illustrated in figure 4, pattern 110 (Miami silt loam). All three Miami samples were calcium-saturated, and the differences resulted from use of different drying mediums and from differences in the degree and uniformity of hydration. In certain fine clays which are lower in montmorillonite, for example, in those from the B₂ horizon of Hagerstown soil[6] from St. Francis County, Mo., and from the B₂ horizon of Fullerton soil[6] from Cherokee County, Ala., the (001) line is clearly brought out by proper control of the hydration, but is very weak or absent in patterns obtained when hydration is not adequately controlled (fig. 4).

The effectiveness of the controlled hydration procedure was further demonstrated when applied in studies of synthetic mixtures of montmorillonite and hydrous mica (6). X-ray diffraction patterns of a number of synthetic montmorillonite-hydrous mica mixtures were prepared to provide a basis for quantitative estimation of montmorillonite and hydrous mica in soil fine clays. A 16 A. montmorillonite line appeared in diffraction patterns of samples containing as low as 5 per cent montmorillonite mixed with 95 per cent hydrous mica.

SUMMARY

To differentiate montmorillonite from hydrous mica and make possible the quantitative estimation of these minerals in clay samples by means of the x-ray diffraction procedure, it is necessary to control the degree of hydration of montmorillonite more closely than previously was thought necessary. Too high or too low hydration of montmorillonite results in diffraction lines at 10 or 12 A. spacing, that is, in the position of characteristic hydrous mica lines. Obviously, it is necessary to avoid the formation of these lines by montmorillonite if the presence and the amount of montmorillonite and hydrous mica in clay are to be judged by their characteristic lines. A procedure for attaining the required hydration control has been developed, the essential features of which follow:

Fine clay (particles less than 0.2 μ in diameter), conditioned and saturated with calcium according to a procedure described in a previous paper, is suspended in a ternary solution of benzene, ethanol, and water, the composition of which is such as to provide true solution but near-saturation with respect to water. Benzene is added to the suspension, resulting in setting free a water-rich second

tion of the figures from the x-ray diffraction films, the central area of the pattern was illuminated more strongly than the remainder of the pattern so as to produce in the print the contrast observed in the film. Numbers in parentheses refer to x-ray diffraction pattern numbers.

I. Hydration controlled at optimal by procedure described, involving hydration prior to drying from ternary solution (largely benzene) and humidification at 92 per cent relative humidity.

II. Hydration partly controlled by drying from benzene and humidification by exposure to laboratory atmosphere.

III. Hydration low and nonuniform, resulting from drying the sample from water and forming oriented flakes or aggregates which resist satisfactory humidification. The results shown for Miami soil are representative of this treatment for other soil clays, patterns for which are not reproduced here.

[6] Soil samples C1486 and C109, respectively, kindly supplied by S. B. Hendricks, of the U. S. Department of Agriculture.

phase in finely divided form throughout the suspension so that it is sorbed uniformly by all of the clay. The amount and composition of the ternary solution in which the clay is suspended, and the amount of benzene later added, are suitably adjusted so as to expel the required quantity of water (50 per cent of the dry weight of the clay) and still maintain a suspension medium which is largely benzene. Most of the supernatant liquid is decanted, and the clay dried under controlled conditions (30°C. and 65 per cent relative humidity).

The clay thus treated dries to an incoherent powder, somewhat softer than that resulting from drying the clay from anhydrous benzene. The advantages of drying from a liquid of low polarity are thus retained and improved upon, even though small quantities of water have been added. Experience has shown that the sample in the incoherent physical condition readily reaches equilibrium at any desired relative humidity. A portion of the dried clay is mounted in the receptacle so as to give a definite volume-weight for x-raying, and then is humidified for about 15 hours in a desiccator maintained at 92 per cent relative humidity and later x-rayed at this humidity. Special equipment for this humidification is described. It was found that a maximum intensity of the characteristic 16 A. (001) diffraction line of montmorillonite is produced by this procedure. Diffraction patterns of a number of representative soil clays show clearly defined montmorillonite lines, even in the diffraction patterns of clays made up largely of hydrous mica and kaolinite.

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SOIL COLLOIDS: II. SEPARATION BY PEPTIZATION[1]

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The importance of that fraction of the soil which is designated by the term "colloids" has been pointed out in a previous paper (1), in which a review of some of the theories on the formation, structure, and behavior of the soil colloidal complex was presented. During recent years, a great deal of research work on this continent has been directed toward a study of the crystalline structure of the inorganic soil colloids or colloidal clay. In the study of soil colloids and of their relationship to the problems of soil fertility, however, the important part played by the organic fraction of the colloidal complex cannot be overlooked.

Mattson (2) has pointed out that "the interaction of the various forms of organic matter with the mineral complex is an important problem which deserves a thorough investigation." The need for further work in this direction has been emphasized by Tyulin (5), who has proposed a method for separating from the soil what are termed "organo-mineral gels," a term used to indicate the peculiar chemical composition of soil colloids. This method appears to offer a somewhat different approach to a number of problems involving soil colloids, but a review of the literature reveals that very little work has been done toward applying the method to a study of the soils of this continent.

By the use of this method, it can be shown that different groups of colloids do actually exist in the soil, and the means of bringing about their separation are such that any changes in the colloids themselves will not be great, since no drastic chemical reactions are involved. According to Tyulin, the soil colloids can be classified into two groups, which he called "electronegative colloids" and "isoelectric colloids." He separated the electronegative colloids, referred to as "group 1," by fractional peptization. After saturation with sodium, they were dispersed in distilled water and thus isolated from the soil. The second group, which Tyulin termed "isoelectric" but which may be weakly electronegative, were separated by dissolutive peptization. The soil residue, after removal of group 1 colloids, was treated with 0.004 N NaOH, which removed part of group 2 (which may be termed "group 2a"). The remainder of this group was not peptized until the soil residue, after separation of group 2a, had the mobile sesquioxides eliminated by treatment with weak hydrochloric acid. Then the second part, termed "group 2b," was peptized in weak alkali.

Tyulin carried the fractionation of groups 1 and 2a still further. Treatment of the colloidal suspension, made alkaline to pH 8.5, with KCl caused a precipitation of much of the material, but considerable organic matter—that fraction

most loosely held on the surface of the colloids—remained in solution, from which it could be precipitated by acidification. This constituted a first humate fraction. By treatment of the material precipitated by the addition of KCl with weak acid, the bond between organic matter and sesquioxides was destroyed, and then, after dispersion in weak alkali and subsequent precipitation with KCl, a second humate fraction was obtained. No further fractionation was attempted, but the soil colloids had been separated into groups 1, 2a, and 2b, and of these, groups 1 and 2a had been subdivided into a first humate fraction, second humate fraction, and residue.

METHOD

Because of the inaccessability of the Russian literature in which most of Tyulin's work has been published, difficulty was encountered in obtaining the details of the method. From the information available and after considerable investigation, the following method was worked out in this laboratory, and has been used in the investigation of a number of Canadian soils.

Saturation of soil with sodium

Weigh 50 gm. air-dry soil (passed through a 2-mm. screen) into a 250-cc. beaker. Add 200 cc. NaCl solution (25 gm. per 100 cc., adjusted to pH 7), stir thoroughly, cover, and let stand overnight. Filter, transfer soil sample to filter paper and continue washing with NaCl solution until filtrate gives no test for calcium. Remove excess NaCl solution with gentle suction. Wash sample with 80 per cent ethyl alcohol (at pH 7) until filtrate gives no test for chlorides. Remove excess alcohol with gentle suction. Transfer soil sample to 600-cc. beaker with distilled water.

Separation of group 1 colloids

Add distilled water to the Na-saturated soil in the beaker to a depth of 9 or 10 cm. Stir thoroughly and let stand 24 hours. Siphon off the upper 8 cm. of suspension and put it in a large bottle labelled "group 1 colloids." Again add distilled water to the soil in the beaker, stir, let stand 24 hours, siphon off the upper 8 cm. of suspension, and add it to that removed 24 hours earlier. Repeat until the supernatant liquid, after standing 24 hours, is practically clear of suspended material. (This will, in most cases, take about 3 weeks.)

Separation of group 2a colloids

Add NaOH solution (0.004 N) to the soil residue (after removal of group 1) to a depth of 9 or 10 cm., stir thoroughly, and let stand 24 hours. Siphon off the upper 8 cm. of suspension and put it in a large bottle labelled "group 2a colloids." Again add 0.004 N NaOH solution and continue as in the separation of group 1 until, after standing 24 hours, the supernatant liquid is practically clear of suspended material. (This will also, in most cases, take about 3 weeks.)

Separation of group 2b colloids

To the soil residue left in the beaker (after removal of group 2a) add about 500 cc. of 0.01 N HCl solution, stir thoroughly, and let stand for 24 hours.

Siphon off as much of the supernatant liquid as possible, and repeat the treatment three to six times until the pH value of the supernatant liquid is the same as that of the 0.01 N HCl added.

Next add 0.01 N NaOH solution to the soil sample to a depth of 9 or 10 cm., stir, and let stand 24 hours. Siphon off the upper 8 cm. of suspension and put it in a large bottle labelled "group 2b colloids." Add distilled water to a depth of 9 or 10 cm., stir, let stand 24 hours, siphon off the upper 8 cm. of liquid, and add it to the liquid removed 24 hours earlier. Repeat these treatments, using 0.01 N NaOH and distilled water alternately until the supernatant liquid, after standing 24 hours, is practically clear of suspended material. (This will, in most cases, take about 2 weeks.)

Treatment of the residue

The residue, after being washed three or four times with distilled water (by stirring, letting settle 24 hours, and siphoning off the supernatant liquid) is dried, weighed, and kept for analysis.

Fractionation of group 1 colloids

Measure the total volume of suspension collected. Mix the suspension thoroughly and remove about one-fourth to one-fifth of the group for examination. To the remainder, add NaOH solution (about 15 cc. 0.1 N NaOH per liter of suspension), shake thoroughly, and let stand overnight. Determine the pH value, which should be between 8.5 and 10.0. If it is less than pH 8.5, adjust it by adding more 0.1 N NaOH.

Add solid KCl (15 gm. per liter of suspension), stir thoroughly, and let stand overnight. Centrifuge to remove all of the material precipitated by the KCl (called the "KCl floc"). Save both the supernatant liquid and the KCl floc. To the supernatant liquid, add HCl (1 + 3) at the rate of 5 cc. per liter to precipitate the first humate fraction. Centrifuge, and keep the humate fraction for analysis.

To the KCl floc, add 0.01 N HCl, stir, and let stand overnight. Centrifuge, decant, and repeat the HCl treatment two to four times until the supernatant liquid has the same pH value as that of 0.01 N HCl. Disperse the KCl floc in NaOH solution (0.01 N), add solid KCl (15 gm. per liter), stir, and let stand overnight. Centrifuge, and keep both the supernatant liquid and the floc.

To the supernatant liquid, add HCl (1 + 3) at the rate of 5 cc. per liter to precipitate the second humate fraction. Centrifuge, and keep the humate fraction for analysis. Wash the KCl floc two or three times with 0.01 N HCl by stirring and centrifuging, and keep it for analysis.

Fractionation of group 2a colloids

Measure the total volume of suspension collected. Mix the suspension thoroughly and remove about one-fourth to one-fifth of the group for examination. To the remainder, add KCl at the rate of 15 gm. per liter. (Since group 2a is dispersed in 0.004 N NaOH, the suspension should have a reaction above pH 8.5.) From this point, continue the fractionation exactly as for group 1.

Fractionation of group 2b colloids

The fractionation of this group is carried out as for group 2a, but only one humate fraction is separated. The soil residue after separation of group 2a is treated with 0.01 N HCl before group 2b is removed, so that a further treatment with HCl after removal of one humate fraction would appear to be unnecessary.

RESULTS

The soil samples selected for study by this method were collected from a number of locations across Canada, and all were of medium texture. They formed a group of seven which were used in greenhouse work in a study of factors affecting the response of crops to applications of phosphatic fertilizer as part of a general investigation of the problem of phosphate fixation. The following brief description of the soils gives the soil zone (3) and the location from which each sample was taken.

Laboratory no. 7443 Gray-brown podzolic, from the Central Experimental Farm, Ottawa, Ont., plot N 4.

7445 Podzol, on carboniferous till, from the Dominion Experimental Farm, Nappan, N. S.

7541 Black soil (chernozem), from the Dominion Experimental Station, Lacombe, Alta., rotation C.

7543 Black soil (chernozem), from the Dominion Experimental Station, Lacombe, Alta., project 512.

8287 Podzol (Appalachian upland), from Macdonald College experimental plots, Sawyerville, P. Q.

8289 Gray wooded, from the University of Alberta experimental plots, Breton, Alta., series E, plot 5.

9823 Dark brown prairie, from the Dominion Experimental Station, Scott, Sask., rotation C, plot 2.

All samples were taken from cultivated areas and represented the soil to plow depth. In all cases, they came from areas which had received no applications of phosphatic fertilizer or, in most cases, fertilizer treatments of any kind. The results of some analyses of these samples are given in table 1. These show that the reactions varied from pH 5.1 to 7.3, the carbon contents from 1.58 to 6.77 per cent, the nitrogen contents from 0.14 to 0.64 per cent, the phosphorus contents from 0.024 to 0.094 per cent, and the base-exchange capacities from 9.6 to 42.6 m.e. per 100 gm. of air-dry soil.

During the early stages of this study (in 1942) 50-gm. samples of each of the seven soils were used. The separation of the groups, especially group 1, was not continued until dispersion was complete but was discontinued when it was thought that the greater part of group 1, enough for examination, had been obtained. At a later date (in 1943) five of the seven samples, together with a number of others, were used for a more thorough investigation. Larger samples were taken, either 200 or 300 gm. depending on the amount of colloids to be expected, and the separation of each group was made as complete as possible. In table 2 are presented the amounts of colloids of the different groups that were separated, as outlined above, in the 2 years.

The first observation to be made from an examination of these figures is the great variation in the amount of group 1 colloids from the different soils. On the basis of the 1942 results, the Lacombe 512 sample yielded 19.3 per cent and the Sawyerville soil only 1.1 per cent. The variation in the amount of group 2a colloids was not so great as that of group 1, and, the amounts of the group 2b colloids were generally small and showed little variation. For the five soils examined more thoroughly in 1943, the amount of group 1 colloids was, in every case, somewhat greater. This was to be expected, since, as pointed out above, the separation of group 1 in 1942 was not carried to completion. The increased

TABLE 1

Analysis of seven selected Canadian soils

LABORA-TORY NUMBER	SOIL	pH	CARBON	NITRO-GEN	PHOS-PHORUS	BASE-EXCHANGE CAPACITY PER 100 GM. SOIL
			per cent	per cent	per cent	m.e.
7443	Gray-brown podzolic, Ottawa	7.3	1.58	0.14	0.070	9.6
7445	Podzol, Nappan	6.1	2.32	0.14	0.024	10.8
7541	Black (chernozem), Lacombe C	5.7	6.75	0.64	0.094	36.0
7543	Black (chernozem), Lacombe 512	6.5	6.77	0.62	0.092	42.6
8287	Podzol, Sawyerville	5.1	3.85	0.31	0.052	12.1
8289	Gray wooded, Breton	6.5	1.67	0.14	0.054	13.3
9823	Dark brown prairie, Scott	6.0	2.68	0.29	0.060	17.4

TABLE 2

Amounts of the different groups of colloids in selected Canadian soils

As per cent of air-dry soil

LABORATORY NUMBER	SOIL	1942 RESULTS			1943 RESULTS		
		Group 1	Group 2a	Group 2b	Group 1	Group 2a	Group 2b
7443	Ottawa	3.5	5.5	4.8	4.6	2.7	3.3
7445	Nappan	4.4	6.8	3.4			
7541	Lacombe C	12.0	13.9	3.8	14.5	11.1	4.4
7543	Lacombe 512	19.3	10.0	4.0	22.5	8.4	4.4
8287	Sawyerville	1.1	6.8	3.7			
8289	Breton	5.7	5.8	3.9	7.3	3.2	2.6
9823	Scott	10.8	8.8	4.1	12.8	5.3	2.8

figures did not affect the order of magnitude of yield of group 1 colloids from the different soils. In 1943, the yields of group 2a colloids were, in every case, less than in 1942. This may have been due to the more complete separation of group 1, which otherwise might be dispersed in the very dilute NaOH solution used to peptize group 2a. There was no regular change in the amounts of group 2b obtained in the two years, but the quantities were small and showed considerably less variation than did the percentages of the other two groups.

Results of the more complete separation carried out on five soil samples in 1943 are presented in table 3. In the group 1 colloids, 85 to 96 per cent of the

total was accounted for by the three fractions; in the group 2a colloids, these figures were 82 to 95 per cent. The recovery of the group 2b fraction was rather poor, the best being only 83 per cent, and, in two cases, the figures drop to approximately 50 per cent. The reason for such a low recovery is not clear.

The first humate fraction, in both groups 1 and 2a, was larger than the second humate fraction. This difference was pronounced in the two Lacombe soils. Tyulin believes that the loosely bound organic matter, as represented by the first humate fractions, plays a very important part in certain soil reactions and properties. In general, the figures in tables 2 and 3 indicate the distribution of the groups of colloids and their fractions that may be expected in a variety of soils.

TABLE 3

Amounts of the fractions of the different groups of colloids in selected Canadian soils

As per cent of air-dry soil

LABORATORY NUMBER	SOIL	GROUP 1			GROUP 2a			GROUP 2b	
		First humate fraction	Second humate fraction	KCl floc	First humate fraction	Second humate fraction	KCl floc	Humate fraction	KCl floc
7443	Ottawa	0.23	0.22	3.5	0.42	0.12	2.0	0.18	2.2
7541	Lacombe C	1.29	0.33	11.9	2.66	0.28	6.9	0.49	2.7
7543	Lacombe 512	1.44	0.50	19.8	1.44	0.20	5.6	0.58	3.1
8289	Breton	0.18	0.11	6.5	0.35	0.06	2.2	0.12	1.3
9823	Scott	0.38	0.29	11.5	0.58	0.08	3.7	0.15	1.2

TABLE 4

Base-exchange capacities of soil samples before and after separation of the colloid groups

As m.e. per 100 gm. of air-dry soil

	OTTAWA	NAPPAN	LACOMBE C	LACOMBE 512	SAWYERVILLE	BRETON	SCOTT
Original soil...............	9.6	10.8	36.0	42.6	12.1	13.3	17.4
After separation of groups..	lost	6.0	17.2	19.3	7.6	6.1	5.2

Determination of the base-exchange capacities of the residues, after separation of the groups of colloids, showed that, in every case, this material still had the power of adsorbing an appreciable amount of cations. The figures for the exchange capacity (ammonia adsorbed by treatment with neutral normal ammonium acetate solution) are presented in table 4. The determinations made on the original soil sample and on the residues are given and indicate that the different soils have retained approximately 30 to 60 per cent of their power to adsorb cations after groups 1, 2a, and 2b have been removed.

DISCUSSION

Results obtained by Tyulin (5) indicated that the group 1 colloids predominate in a typical chernozem soil but that group 2 colloids form the greater fraction

in a red soil and also in a podzol. It was evident from the results obtained in this laboratory that the ratio of group 1 to group 2 may depend on the completeness of the separation of group 1. The bulk of each group was removed early in the separation (after seven to ten periods of 24 hours), but smaller quantities continued to remain in suspension after 24 hours' standing up to more than 3 weeks from the time peptization was begun. Thus, in 1942, when dispersion of group 1 colloids and removal of the supernatant liquid after 24 hours was repeated approximately 10 times, the percentage of group 1 colloids was less in every case than in 1943, when the supernatant liquid was removed over 20 times. Conversely, the quantity of group 2a was less in every case in 1943 than in 1942. The only directions obtainable on this point were that the supernatant liquid is removed every day "until the layer of liquid over the deposit is clear" (4) or that "the operation is stopped when the supernatant liquid begins to get clear" (7). It is possible that, if the treatment is prolonged, especially in the separation of the second group when weak alkali is used, changes may be brought about in the nature of the colloid or substances brought into solution, e.g., by hydrolysis, which otherwise might not be dispersed.

Recent attempts by Russian workers (6, 7) have been directed toward obtaining a rapid method of bringing about the separation of the colloid groups from the soil, based to some extent on the method for mechanical analysis. This would indicate that the more complete separation that was obtained in the time-consuming method outlined in this paper is probably not necessary to give a satisfactory picture of the colloid distribution. Furthermore, results obtained in this laboratory (to be published in a later paper) have indicated that it is probably the group 1 colloids that play the most important part in the general question of soil fertility, and the figures presented in the present paper show that the relative amounts of this group from the soils used were not changed when the more complete separation was attempted.

On the basis of the 1942 results, taking the sum of groups 2a and 2b to obtain a value for group 2, the Nappan podzol had 4.4 per cent group 1 and 10.2 per cent group 2 colloids, and the Sawyerville podzol had only 1.1 per cent group 1 and 10.5 per cent group 2. These results agreed in general with those obtained for a podzol by Tyulin. On the other hand, only one of the two Lacombe soils from the black soil zone, closely resembling chernozem, had a greater amount of group 1 than group 2 colloids. An interesting observation made on the Sawyerville podzol was that the first humate fraction obtained from group 2a constituted about 50 per cent of that group, or over 3 per cent of the original soil, an amount greater than the total yield of group 1 colloids from this soil.

SUMMARY

An attempt has been made to fractionate the colloids of a number of soils into groups as proposed by Tyulin. Based on information available, a method has been worked out for the separation of group 1 colloids (called by Tyulin the "electronegative" group) and of group 2 colloids (Tyulin's so-called "isoelectric" group), the latter being obtained as two subgroups. Group 1

includes those colloids that are dispersed in distilled water after being saturated with Na ions. Group 2, subgroup "a," contains those colloids which, following the removal of group 1, are dispersed in 0.004 N NaOH. Group 2, subgroup "b," consists of those that are dispersed in weak alkali following the removal of group 2a and subsequent treatment of the residual soil with weak acid. From each group have been separated a first humate fraction, representing loosely bound organic matter, and a second humate fraction, or organic matter more firmly attached to the colloids. Details of the methods as used in this laboratory have been given, and some results obtained on soils of medium texture, taken from different soil zones, have been presented.

It has been possible to obtain the various groups and their fractions as indicated by Tyulin, and these have varied in amount from soil to soil. The greatest variation was shown in the size of the group 1 fraction, from 1.1 to 19.3 per cent. The variation in the quantities of group 2a was less, and the percentages of group 2b were in general the smallest and showed the least variation between soils. Results have indicated that the relative quantities of group 1 and group 2 may depend on the completeness of the separation of the first group. It has been suggested that, in order to get comparable results, it may not be necessary to make the separation of the different groups as complete as indicated in the method given. It has been shown that the residues, after separation of the groups, have retained from 30 to 60 per cent of the capacity to adsorb bases possessed by the original soil.

In agreement with Tyulin's results, the podzols examined, especially that from Sawyerville, had considerably more of group 2 than of group 1 colloids. One of the Lacombe samples, from the black soil zone, had a larger percentage of group 1, as found by Tyulin for a typical chernozem.

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BOOKS

Emulsion Technology. Chemical Publishing Company, Inc., Brooklyn, 1943. Pp. 290. Price $5.

This book is made up of a series of papers prepared by specialists in the field of emulsion technology. Both the theoretical and the applied aspects of emulsions are presented. The several chapters deal with mechanisms, principles, uses, patents, machinery, and problems of emulsification, and with applications in insecticides, fungicides, foods, textiles, paints, leathers, rubbers, and asphalts. The contributing editors are W. R. Atkin, N. H. Chamberlain, W. Clayton, R. M. K. Cobb, J. W. Corran, F. G. Donnan, R. Dorey, A. C. Fraser, H. Freundlich, L. G. Gabriel, M. P. Hofmann, R. I. Johnson, L. A. Jordan, J. B. Speakman, H. P. Stevens, W. H. Stevens, F. C. Thompson, V. G. Walsh, S. Werthan, and R. M. Woodman.

An Introduction to Pollen Analysis. By G. Erdtman, with a foreword by Robert P. Wodehouse. Chronica Botanica Company, Waltham, Massachusetts, 1943. Pp. 294, figs. 48. Price $5.

This is Volume XII of "A New Series of Plant Science Books." It stresses the importance of pollen morphology and presents a set of theoretical principles based on the experimental work so far undertaken. The science of pollen analysis is relatively young but, judging from this volume, a great deal of progress has been made in it. The results of pollen analyses are of particular interest to ecologists, climatologists, geographers, and historians. As would be expected, a large part of the book is taken up with a consideration of peat and of the pollen grains that have been preserved in such deposits. Anyone interested in this subject will find this book very useful.

Plant Viruses and Virus Diseases. By F. C. Bawden. Second edition, entirely revised. Chronica Botanica Company, Waltham, Massachusetts, 1943. Pp. 294, figs. 48. Price $4.75.

This is Volume XIII of "A New Series of Plant Science Books." Among the 16 chapters contained in it are those dealing with the history, symptomatology, transmission, vectors, strains, serological reactions, purification, properties, methods of inactivation, classification, and control of viruses. One is impressed with the progress that has been made in this line of work, and this progress has been faithfully and fully reported. One is impressed also with the large amount of effort which must still be put into the investigation of this intriguing subject.

THE EDITORS.

SOIL COLLOIDS: III. RELATIONSHIP TO SOIL FERTILITY[1]

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The practical application of any study on soil colloids should be found in the relationship of these colloids to soil fertility. It is generally agreed that the colloidal complex of a soil plays a major part in supplying nutrients to plants grown thereon. There is still a need for some method of relating soil fertility more closely with some property or some fraction of the colloidal complex. Attention has been drawn recently (2) to the importance of the clay-humus complexes or so-called "organo-mineral" colloids in soil fertility, with particular reference to the work of Tyulin in Russia and of Simon and Springer in Germany.

The investigations of Tyulin (4) are based on the separation of soil colloids by fractional and dissolutive peptization into group 1 (called "electronegative" colloids) and group 2 (termed "isoelectric" by Tyulin and obtained in two subgroups), and the further treatment of these groups to obtain a first humate fraction representing loosely bound organic matter, and a second humate fraction representing organic matter more firmly attached to the colloids. A previous paper (1) reported results obtained from an attempt to apply Tyulin's method to the fractionation of the colloids of a number of Canadian soils. The method proved to be generally applicable, and some of the fractions varied considerably in quantity from soil to soil. The greatest variation was shown in the amounts of group 1 colloids; the variation in the quantities of group 2a was somewhat less; and the amounts of group 2b were small and showed little variation.

While the laboratory experiments were being conducted, greenhouse studies on the same soils were also being made. Large samples of the untreated soils from the different locations were brought to Ottawa so that studies of fertility and the effect of fertilizer treatments, particularly applications of phosphatic fertilizer, could be undertaken under the same environmental conditions. This arrangement made it possible to see whether any relationship existed between the inherent fertility of a soil sample and the yield of colloidal fractions obtained by Tyulin's peptization method.

A number of fertilizer treatments were applied in the greenhouse to each soil, but it is necessary here to consider only the yields of the untreated samples. In the fall of 1940, the samples in an air-dry condition were placed in flower pots, an amount equivalent to 2000 gm. of moisture-free soil being used per pot. Barley of the variety O.A.C. 21 was used as the indicator crop, and seven seeds per pot were sown but, after germination, only four plants per pot were allowed

to grow. Each treatment was made in triplicate. In May, 1941, the plants were harvested and the yields determined.

After the harvesting of the plants, no more water was added, and the soils were kept in an air-dry condition throughout the summer. In the fall of 1941, the soils were cultivated and moistened. As in the previous year, barley (O.A.C. 21) was grown at the rate of four plants per pot and the yields were again determined after harvesting.

During the third year, the whole experiment was rearranged somewhat, and red clover was grown instead of barley. The soil from Sawyerville was given special treatments, with barley again as the indicator crop, but clover was grown on two pots immediately following the barley.

TABLE 1

*Relationship between plant yield and the amount of nitrogen, of carbon, and of group 1 colloids in the soil**

SOIL	NITROGEN IN SOIL	CARBON IN SOIL	GROUP 1 COLLOIDS IN SOIL	YIELD OF BARLEY GRAIN PER POT, 1940–41	YIELD OF BARLEY GRAIN PER POT, 1941–42	YIELD OF CLOVER PER POT, 1942–43
	per cent	per cent	per cent	gm.	gm.	gm.
Lacombe 512................	0.62	6.77	19.3	4.9	8.4	21.5
Lacombe C.................	0.64	6.75	12.0	4.7	7.2	14.7
Scott.......................	0.29	2.68	10.8	6.9	3.6	14.0
Breton......................	0.14	1.67	5.7	3.9	1.8	13.1
Nappan.....................	0.14	2.32	4.4	3.9	2.6	7.0
Ottawa.....................	0.14	1.58	3.5	2.1	2.0	3.8
Sawyerville.................	0.31	3.85	1.1	0.7	1.0	4.2†

* *t* value, group 1 colloids and barley yield, 12.0
  t value, per cent nitrogen and barley yield, 5.6
  t value, per cent carbon and barley yield, 5.2
  Necessary *t* (*P* = 0.01, *n* = 17), 2.90
  t value, group 1 colloids and clover yield, 8.6
  t value, per cent nitrogen and clover yield, 4.5
  t value, per cent carbon and clover yield, 4.1
  Necessary *t* (*P* = 0.01, *n* = 24), 2.80
† Average yield from two pots, immediately after barley.

Three cuttings of clover were made over a period of several months in the spring and summer of 1943. The total yield was determined by adding together the weights obtained at each cutting. The yields of barley for two years and of clover for one year are presented in the last three columns of table 1.

From a study of the results obtained from the fractionation of the colloids, a reasonably close agreement appeared to exist between the yields obtained in the greenhouse and the amount of group 1 colloids. The figures for the latter are also presented in table 1 for the purpose of comparison.

In 1940–41, the yield of barley from the Scott soil was greater than that from either of the Lacombe soils, which contained a greater amount of group 1 colloids. This result was rather unexpected, since field data on wheat production

from these soils showed that the Lacombe soils gave higher yields. At each location, the rotation of summer fallow, wheat, wheat, was followed, and the following figures are the yields of wheat after summer fallow:

Lacombe 512 Average yield, 1935–1941, 36.8 bushels per acre.
Lacombe C   Average yield, 1935–1941, 21.4 bushels per acre.
Scott   Average yield, 1935–1941, 9.0 bushels per acre.

The greenhouse yields of barley for 1941–42 showed that, for the three soils just discussed, the order of yield was the same as that for the quantity of group 1 colloids. In this year, however, the yield from the Breton soil appeared to be somewhat out of line. Otherwise, the yield figures agreed in order of magnitude with those for the first group of colloids. When clover was used as the indicator crop in 1942–43, even better agreement was obtained. The yield of clover increased in the same order as the quantity of group 1 colloids separated from the soil.

A statistical analysis of the data was made to determine the correlation between the amounts of group 1 colloids and the yields obtained. The total yield of barley for the two years from each individual pot in the experiment was used, and it was found that $r = +0.946$ with a t value of 12.0 and necessary t for significance, when $P = 0.01$ and $n = 17$, of 2.90. When the yield of clover from each pot in the series was used, it was found that $r = +0.85$ with a t value of 8.6 and necessary t for significance, when $P = 0.01$ and $n = 24$, of 2.80. Both results indicate highly significant correlations.

When the yields of barley and of clover were compared with the nitrogen content and the carbon content of the soil, highly significant correlations were also obtained. The t values obtained were 5.6 for nitrogen and barley, 5.2 for carbon and barley, 4.5 for nitrogen and clover, and 4.1 for carbon and clover, but these were very much lower than those obtained when the yield was compared with the amount of group 1 colloids. The two Lacombe soils had almost identical amounts of organic matter ($N = 0.62$ per cent and 0.64 per cent; $C = 6.77$ per cent and 6.75 per cent), but the yields of barley and, more particularly, of clover were lower on the Lacombe C sample, which had the smaller amount of group 1. A comparison of the Scott and Sawyerville samples is also interesting. The Sawyerville soil had slightly more organic matter (0.31 per cent N and 3.85 per cent C) than the Scott soil (0.29 per cent N and 2.68 per cent C). The amount of group 1 colloids, however, was very much greater in the Scott sample (10.8 per cent) than in the Sawyerville sample (1.1 per cent), and the yields of barley and of clover were considerably greater for the Scott soil.

Shchukina (3) has shown that the improvement in yield capacity on an irrigated chernozem was reflected in the amount of group 1 colloids. Tyulin (4) has pointed out the preponderance of group 1 colloids in a chernozem (a relatively fertile soil) when compared with the same group in a podzol (a relatively infertile soil). The few results presented herewith, obtained from a study of soils of medium texture from a number of soil zones in Canada, also have suggested the importance of the amount of group 1 colloids in soil fertility. Tyulin

expressed the opinion that it is the quantity of loosely bound humate fraction of group 1 colloids that may serve as a valuable criterion of soil fertility and quoted some results from his own laboratory in support of this. The quantities of first humate fraction in five of the seven soils mentioned in this paper were presented in a previous publication (1). These results indicate that the agreement between yield and the quantity of this humate fraction was sufficiently close to warrant further investigation. Some preliminary work with two clay soils which are now under study also has pointed to the possibility that this humate fraction may be of more importance than group 1 as a whole, and the study is being continued.

SUMMARY

Greenhouse studies for three years were made on seven Canadian soils of medium texture, representing five soil zones. Yields of barley for two years and of clover for one year were measured. A highly significant relationship was found between the yields and the quantity of group 1 colloids obtained from the soils. This relationship was somewhat closer than that between yields and percentage of nitrogen in the soil, or than that between yields and percentage of carbon. Tyulin's contention that it is the first humate fraction of group 1 colloids that is the important one from the point of view of soil fertility was shown to have support.

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CHANGES IN THE CONTENT OF CERTAIN B-VITAMINS IN ORGANIC MATERIALS DECOMPOSING UNDER AEROBIC AND ANAEROBIC CONDITIONS[1]

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There is evidence that significant amounts of accessory growth substances are present in soils. Whether or not they significantly affect development of crop plants, however, or can do so is still open to speculation. There are some who consider that the effects of the soil-contained vitamins on higher plants are negligible, but others are inclined to believe that crop residues, manures, and other organic materials exert effects on plants not alone through their content of fertilizing elements but also through the vitamins and other accessory growth substances which they contain or which are produced during decomposition of the organic materials by the soil microorganisms.

The experiments reported in this paper were designed, not to test either of these points of view, but rather to obtain information regarding the related subject of the effects of microorganisms on the vitamin content of decomposing organic materials. It was thought that this information would provide a basis for a more reasonable evaluation of the vitamin-supplying potentialities of plant residues.

Very little is known concerning the resistance of vitamins to microbial attack. When vitamins are used in culture media it is generally assumed that they persist or are absorbed by the organisms being cultured and are utilized as growth accessories. Likewise, vitamins have been added to soil in plant tests with no regard to the possibility that they might be decomposed. A point of view recently expressed by Schopfer (45, p. 225) is typical: "The roots of decomposing plants give up their growth factors to the soil just as does the decaying foliage . . . .Decomposing animal organs must also give up their vitamins to the soil."

The results reported here indicate that this is not an accurate statement of what happens during decomposition. The importance of microorganisms in the production of vitamins in the digestive tracts of ruminants and other animals has been emphasized, but little consideration has been given to the possibility that microorganisms may also be responsible for the destruction of vitamins in these organs. Many questions arise in considering the fate of vitamins in plant materials. Are vitamins resistant to microbial attack? What happens to the vitamins contained in decomposing plant materials? Does the vitamin content drop, remain constant, or increase? Since vitamins may be synthesized during microbial development, the vitamin content of a substrate will be determined not only by the rate of decomposition but also by the rate of vitamin synthesis. Thus,

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there is possibility of increase as well as decrease. Studies of the effects of mixed microbial populations on vitamin changes in decomposing plant materials should give some indication of the relative importance of microorganisms in determining the vitamin content of soils and might provide some suggestion as to the likelihood of higher plants obtaining vitamins from the soil.

REVIEW OF LITERATURE

Vitamins in Soil

Since many if not all of the vitamins of the B-complex are required by most living cells and since soils contain the residues of higher plants as well as an extensive and varied population of microorganisms, it is to be expected that the vitamins occur in soils. Lilly and Leonian (26) detected the presence of thiamine and its moieties and also biotin in a large number of soils; larger quantities of these substances were found in the surface than in the deep soil layers and there appeared to be some correlation between the organic matter content and the amounts of vitamins in the soils. Müller (34) likewise obtained evidence that thiamine is present in soils of forests, fields, and prairies, with the greatest amounts in the surface material. Carpenter (11) recovered riboflavin from soils and also noted correlations between the vitamin content and the organic matter content of the soils. Auxins, which are of interest as growth regulators of higher plants and, like vitamins, are effective at very low concentrations, also occur in soils (35, 52); larger quantities were present in fertile soils, and the amounts were close to concentrations that have been found to favor plant development. Auxins are produced by many different soil microorganisms, particularly the bacteria (37).

West obtained evidence that the soil vitamins may come not only from dead plant wastes and microorganisms but also from living plants (57). He found that thiamine and biotin were excreted by the roots of seedlings. Of still greater interest is the finding of West and Lochhead (58, 59) that the microbial population of the rhizosphere includes many bacteria which require preformed accessory growth substances. Organisms of this type occur throughout the soil but are more abundant in fertile soils and in contact with plant roots. This indicates that vitamins are present in soil and that some of the soil microorganisms depend on associating organisms for accessory growth substances. The fact that soil extract provided growth accessories necessary for development of these bacteria proved that the substances were actually present in the soil. From studies using soil extract in culture media, Lochhead and Chase (27, 28) obtained evidence that some soil bacteria require certain growth substances in addition to the known vitamins of the B-complex.

Decomposition of vitamins

The quantity of a vitamin in soils and composts is determined not only by the amounts added in plant residues or formed by microorganisms during decomposition of the organic matter, but also by the extent to which the vitamins themselves are attacked and destroyed by microorganisms. In a previous communication the author reported that, during decomposition of organic matter in composts, there was considerable decomposition of riboflavin and pantothenic acid (51). In fact, it was noted that the vitamin loss was generally greater than that of the total organic matter after a month or more of composting. Consequently, the final products of composting were poorer in the vitamins than were the original plant materials. Similar results were obtained with organic materials that decomposed rapidly as well as with resistant plant residues. Most of the determinations were made after the materials had composted for a month or more, and little information was obtained about changes that took place in the early stages of decomposition.

Kraus (24) found that the riboflavin content of sewage sludge increased during the early stages of digestion but that it decreased during more prolonged anaerobic digestion. Selye (46) reported that riboflavin was rapidly destroyed in certain segments of the intestinal

tract of the rat. Some specific information about the nature of the breakdown of ribo-flavin was recently reported by Foster (17). He isolated from soil a hitherto undescribed bacterium, *Pseudomonas riboflavinus*, which decomposed riboflavin rapidly, oxidizing the ribose group of the molecule completely to CO_2 and H_2O, and leaving intact the rest of the molecule, lumichrome. His results suggest that there may be a high degree of specificity among organisms with regard to the decomposition of vitamins. Young and James (63) and Young and Rettger (64) found that vitamin C is decomposed by a large number of in-testinal bacteria. There are many bacteria, however, that do not attack the vitamin. As a result of microbial attack, the molecule was appreciably altered and transformed beyond the reversible dehydro state.

Some idea of the decomposability of vitamins can be derived from studies on silage and foods that are acted upon by microorganisms during processing. Such information, how-ever, is little more than suggestive as regards transformations in soil, because comparatively simple populations of specific microorganisms bring about the changes in foods. Some results on cheese are of interest in this connection, however. Sullivan, Bloom, and Jarmol (54) found that there was a progressive increase in the amounts of pantothenic acid, nico-tinic acid, and biotin in limburger cheese during the process of curing. Burkholder, Collier, and Moyer (10) found that there was an increase in the amounts of thiamine, riboflavin, nicotinic acid, and biotin in the surface portion of various cheeses during curing, and the increase was ascribed to microbial synthesis. The vitamin content within the cheese changed comparatively little. The persistence of the vitamins was remarkable in view of the results reported below. It may be that the explanation is to be found in the fact that the selected microbial population of the cheeses had relatively few vitamin-decomposing organisms.

EXPERIMENTAL METHODS

Composts composed of organic matter alone were used in order to avoid inter-fering effects of soils. Thus, the vitamin contents of water extracts were suffi-ciently concentrated for direct assays; further concentration might have been necessary if mixtures of soil and organic matter had been prepared. Further-more, any absorption or inactivation of vitamins by soil was also avoided. Three of the vitamins of the B-complex were studied: riboflavin, pantothenic acid, and nicotinic acid. The assays were made by bacteriological methods using *Lactobacillus casei* as the test organism for riboflavin according to the method of Snell and Strong (48) and for pantothenic acid by the method of Strong, Feeney, and Earle (53). Nicotinic acid was determined by the method of Snell and Wright (49) using *Lact. arabinosus* as the test organism.[2]

The extracts used for making the assays for nicotinic acid and pantothenic acid were prepared as follows: The organic material was suspended in distilled water, neutralized, and heated at 1 atmosphere pressure for 30 minutes. The mixture was cooled, made to volume, and centrifuged. The supernatant liquid was used for the assays. In preparing the riboflavin extracts the organic mate-rials were treated with $0.1N$ HCl instead of distilled water.

The first experiments are concerned with an extension of a previous report (51) on the transformation of riboflavin and pantothenic acid in composting or-ganic materials. The first of those recorded below are concerned with changes in

the nicotinic acid content of the same composts.   The composts were prepared as follows:

1. Oat straw—1,000 pounds of straw, treated with 34 pounds of $(NH_4)_2SO_4$, 10 pounds of superphosphate (20 per cent P_2O_5), and 30 pounds of lime.
2. Oat straw and clover—700 pounds of straw and 300 pounds of clover.
3. Corn stover—1,000 pounds of corn stover treated with the same materials as straw.
4. Silage corn—1,000 pounds of silage corn treated with the same materials as straw.
5. Cow manure (straw bedding).
6. Leaves and timothy hay—700 pounds of leaves and 300 pounds of hay.
7. Woody tree trimmings and leaves—500 pounds of woody residues and 500 pounds of leaves treated with materials at one-half the rate used on straw.
8. Salt hay—1,000 pounds of hay treated with the same materials as straw.
9. Lowmoor peat and timothy hay—700 pounds of peat and 300 pounds of hay.

Each compost contained the equivalent of 1,000 pounds of dry organic matter. All were stored in the open on a platform.   Liquid material that drained from the composts was returned to the top.   Water was added as needed to keep the organic matter moist.   The composts were turned each month, and the samples were taken for analysis at the time of turning.   The composts were prepared during the summer and fall.   The leaves were mature fallen leaves of the hardwoods, maple, oak, and ash.   The woody tree timmings were small branches of maple, oak, and ash less than 2 cm. in diameter and cut in pieces not more than 10 cm. long.   The amount of decomposition at each assay period was estimated from determinations of ash content.

Additional experiments are reported on small composts which were incubated in the laboratory where it was possible to control the environmental conditions and to obtain more representative samples than was possible with the large composts.   Determinations were also made more frequently on the small composts, whereby the course of the changes was followed more closely, particularly during the first few weeks.   For these composts, organic material having the equivalent of 125 gm. oven-dry weight was prepared in glazed earthenware pots.   Three different substances were used: wheat straw, young grass, and a mixture of equal parts of straw and grass.   To each of the pots of straw was added 5 gm. $(NH_4)_2HPO_4$, 1 gm. KCl, and 5 gm. $CaCO_3$.   One pot of each material was kept moist, but not wet, and will be referred to as "aerobic." Similar portions were treated with large amounts of water and were kept at moisture contents between 90 and 95 per cent.   These are referred to as "anaerobic" but, since they were kept in containers that were merely covered but not sealed to exclude air, there was opportunity for aerobic decomposition at the surface.   All composts were kept at 28°C.

At each sampling period, the entire contents of each pot were thoroughly mixed before sampling.   Some difficulty was encountered in obtaining suitable samples from the anaerobic material, since the solid substance was not uniformly dispersed through the liquid.   Reasonably representative samples were obtained by separating most of the liquid from the solid materials at each sampling period and taking suitable aliquots of each for analysis.

The weights of the composts in the pots were determined at each period and samples were taken for determinations of moisture and ash. The amount of decomposition was calculated on the basis of decrease in ash-free organic matter, allowance being made for samples removed. The vitamin content was also calculated on the basis of oven-dry, ash-free organic matter. The ash constituents increased materially during decomposition, exceeding 30 per cent of the total material in some cases, and the results would show somewhat less than the actual changes in vitamin content unless they were calculated on the ash-free basis.

EXPERIMENTAL RESULTS

Transformation of nicotinic acid in outdoor composts

Changes in the nicotinic acid content of the large composts which were exposed to the weather are shown in table 1. The amounts of this vitamin in the materials used in preparing the composts differed considerably. The peat with 2.4 γ per gram was the lowest and the silage corn highest with 29.5 γ. The substances were for the most part low-grade plant materials containing large amounts of structural substances and therefore were poor in vitamins which occur in greatest abundance in the actively growing parts of plants. Assays were made of various other materials, including the following which serve to indicate the range of nicotinic acid content in plant materials and related substances: yeast extract 252, young grass 85, fresh thrashed pea vines 49, pea vine silage 1 year old 21, and a fertile field soil 0.35 γ per gram of dry material. In general, the nicotinic acid content of the various materials tested was two to more than three times as high as that of riboflavin or pantothenic acid.

The changes in nicotinic acid were similar to the previously reported changes in riboflavin and pantothenic acid, but the rate of nicotinic acid loss was generally somewhat slower than that of the other two vitamins. There was a progressive loss of vitamins with no tendency for accumulation after 30 days of composting. In most cases the vitamin content of the composted material (micrograms per gram) was less than that of the initial substance. There are a few exceptions but only three cases where there were appreciable increases in nicotinic acid content during decomposition: the straw and corn stover at the 30-day period and silage corn at 7 days. In three other cases there were insignificant increases in nicotinic acid content in the composting materials. In contrast, in 15 of the 21 cases there was a decrease in the nicotinic acid content of the composting organic matter. This indicates that in general there was more rapid loss of the vitamin than of the organic matter as a whole. This is shown from comparison of the figures in the last two columns of the table.

The course of the changes was similar with readily decomposed materials such .as corn stover, straw and clover, and leaves and hay and with more resistant materials such as straw, manure, and leaves and wood. The values for percentage decomposition of total organic matter of the peat and hay compost appear to be too high; this is particularly the case with the value for the 30-day

TABLE 1

Transformation of nicotinic acid in composts

MATERIAL	ASH ON OVEN-DRY BASIS	NICOTINIC ACID CONTENT			LOSS during composting	DECOMPOSITION OF TOTAL ORGANIC MATTER
		As analyzed	On oven-dry, ash-free basis	Calculated, assuming no vitamin decomposition, oven-dry and ash-free basis		
	per cent	*γ/gm.*	*γ/gm*	*γ/gm.*	*per cent*	*per cent*
Oat straw						
Compost, initial......	11.1*	9.0	10.4	10.4	..	..
Compost, 30 days...	12.5	9.4	11.5	12.0	4	13
Compost, 70 days...	16.8	8.2	10.5	17.3	39	40
Compost, 110 days...	22.3	7.8	10.7	24.2	56	57
Oat straw and sweet clover						
Oat straw...........	6.8	9.0	10.4	....	..	..
Sweet clover.........	6.8	18.1	21.4	....	..	..
Compost, initial......	6.8	11.7	13.7	13.7	..	..
Compost, 30 days...	8.0	9.7	11.3	16.3	31	16
Compost, 70 days...	13.3	9.2	11.4	28.5	60	52
Compost, 110 days...	15.4	9.2	11.9	34.3	65	60
Corn stover						
Corn stover, initial...	10.5*	10.9	12.4	12.4	..	..
Compost, 30 days....	17.3	16.0	20.5	22.1	7	44
Compost, 70 days....	32.7	9.0	14.0	51.7	73	76
Silage corn						
Silage corn, initial...	8.6*	26.2	29.5	29.5	..	..
Compost, 7 days.....	14.3	34.0	42.3	51.8	18	43
Cow manure						
Manure, initial.......	20.0	13.2	17.7	17.7	..	..
Compost, 30 days...	26.1	8.2	11.8	24.9	53	29
Compost, 70 days...	34.1	10.3	16.6	36.9	55	52
Compost, 100 days...	37.1	8.6	14.5	42.1	66	58
Leaves and timothy hay						
Leaves..............	12.3	11.1	13.7	....	..	..
Hay................	5.4	20.2	23.0	....	..	..
Compost, initial......	10.2	13.8	16.5	16.5	..	..
Compost, 30 days...	13.2	10.0	12.3	22.0	44	25
Compost, 111 days...	17.8	6.3	8.5	31.7	73	48
Compost, 163 days...	21.7	7.4	10.3	40.2	74	59
Compost, 252 days...	30.0	7.4	11.5	91.7	87	82

* Includes ash and fertilizer salts.

TABLE 1—*Continued*

MATERIAL	ASH ON OVEN-DRY BASIS	NICOTINIC ACID CONTENT				DECOMPOSITION OF TOTAL ORGANIC MATTER
		As analyzed	On oven-dry, ash-free basis	Calculated, assuming no vitamin decomposition, oven-dry and ash-free basis	Loss during composting	
	per cent	γ/gm.	γ/gm.	γ/gm.	per cent	per cent
Leaves and woody tree trimmings						
Leaves...............	12.3	11.1	13.7	....	..	..
Woody tree trimmings.............	6.8	4.6	5.3	....	..	..
Compost, initial......	11.6*	7.9	9.5	9.5	..	..
Compost, 30 days...	16.7	5.9	7.6	14.4	47	34
Compost, 264 days...	23.5	4.3	6.1	22.1	72	57
Salt hay						
Salt hay, initial......	11.1*	15.9	18.4	....	..	......
Compost, 30 days...	9.1	10.6	12.3	....	33	negligible
Compost, 70 days...	10.8	7.7	9.2	....	50	negligible
Compost, 120 days...	10.4	8.6	10.3	....	44	negligible
Lowmoor peat and timothy hay						
Peat.................	13.3	1.7	2.4	....	..	..
Hay.................	5.4	20.2	23.0	....	..	..
Compost, initial......	10.9	7.3	8.6	8.6	..	..
Compost, 30 days...	17.5	3.5	4.7	14.8	68	42
Compost, 70 days...	20.4	4.0	5.0	17.9	72	52
Compost, 120 days...	21.6	4.7	6.7	19.5	66	56

* Includes ash and fertilizer salts.

period. It is believed that the discrepancy is due to faulty sampling, whereby material having ash content greater than that representative of the compost as a whole was taken for the analyses.

These results and those previously reported (51) give no indication that well-composted material becomes enriched in B-vitamins through microbial action. On the contrary, they indicate that the vitamins are readily decomposed and that a large portion of the vitamin content of plant materials disappears during attack by mixed populations of microorganisms under aerobic conditions.

Effects of aerobic and anaerobic conditions on vitamin transformations

The six small composts incubated in the laboratory were studied next. The aerobic organic materials acquired a musty odor. Within a week, considerable amounts of ammonia were evolved by the grass and the straw-grass mixture due to the high nitrogen content of the grass from which the composts were prepared. Decomposition was rapid in all cases but more so with the aerobic materials; the grass decomposed most rapidly, and the straw, most slowly. The anaerobic

materials showed various evidences of active decomposition: all had putrid odors, the odor of volatile organic acids was noticeable in the straw, and gas evolution was prominent in all three materials but in the straw there appeared to be more gas and the period of active gas production was prolonged. The anaerobic straw had a bleached, fresh-straw color throughout the experiment except for the material at the surface which was in contact with the air. Even

TABLE 2

Changes in vitamin content of decomposing straw

TIME	ASH CONTENT DRY BASIS	RIBOFLAVIN*			PANTOTHENIC ACID*			NICOTINIC ACID*		
		From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition	From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition	From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition
days	*per cent*	γ	γ	γ	γ	γ	γ	γ	γ	γ
Aerobic										
0	9.1	2.0	2.0	0	2.5	2.5	0	8.6	8.6	0
1	10.6	2.9	2.0	0.9	2.7	2.5	0.2	10.0	8.6	1.4
7	11.2	11.3	2.3	9.0	2.8	2.8	0	32.3	9.9	22.4
20	15.5	6.9	3.2	3.7	4.3	4.1	0.2	24.5	14.1	10.4
44	18.0	6.2	3.9	2.3	4.7	4.9	−0.2	20.3	17.0	3.3
72	24.4	6.4	5.6	0.8	5.9	6.9	−1.0	32.0	24.3	7.7
127	27.2	4.0	6.4	−2.4	3.1	7.9	−4.8	18.7	27.9	−9.2
153	27.2	3.1	6.4	−3.3	5.0	8.0	−3.0	16.3	28.2	−11.9
167	27.7	...	...	.....	...	...	.....	19.0	28.7	−9.7
Anaerobic										
0	9.1	2.0	2.0	0	2.5	2.5	0	8.6	8.6	0
1	9.3	2.7	2.0	0.7	0.14	2.5	−2.36	9.5	8.6	0.9
7	9.7	2.0	2.1	−0.1	0.80	2.6	−1.8	4.2	9.1	−4.9
20	9.9	2.1	2.1	0	1.1	2.7	−1.6	4.1	9.3	−5.2
44	11.1	1.9	2.4	−0.5	2.7	3.0	−0.3	9.0	10.5	−1.5
72	13.6	2.9	3.3	−0.4	1.2	4.1	−2.9	7.4	14.3	−6.9
127	17.5	4.0	4.7	−0.7	1.3	5.8	−4.5	7.8	20.5	−12.7
153	17.6	3.3	4.3	−1.0	1.2	5.3	−4.1	7.3	18.7	−11.4
167	20.5	...	...	.....	...	...	.....	11.2	24.5	−13.3

* Per gram of dry and ash-free material.

† Calculated from loss of total organic matter during decomposition.

this darkened material became bleached after being mixed with the submerged substance. The organic materials that were kept moist and aerobic had none of the offensive odors that characterized the anaerobic materials.

Some of the results obtained with the straw compost are presented in table 2 and figure 1. Three kinds of data are shown in the figure: the percentage decomposition of the total organic matter exclusive of the ash constituents; the amounts of riboflavin, pantothenic acid, and nicotinic acid in the ash-free organic matter at each assay period; the percentage increase or decrease in vitamin

content at each assay period in terms of the initial vitamin content. The incubation periods for the graphs in the right hand portion of the figure are the same as those in the table. The columns which are directly above one another refer to the same incubation periods.

The straw decomposed appreciably faster under aerobic conditions than under anaerobic conditions, and the most rapid loss occurred during the first month or two. The aerobic straw showed little change after 80 days, whereas the anaerobic straw continued to show considerable loss even after 120 days and it was evolving gas in appreciable amounts after incubating for well over 7 months.
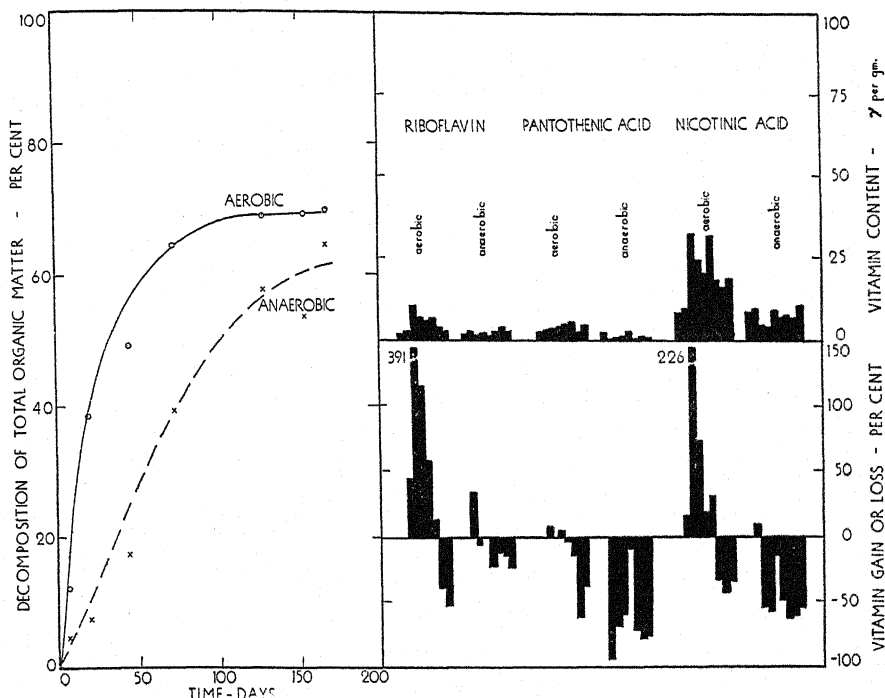

FIG. 1. CHANGES IN THE VITAMIN CONTENT OF DECOMPOSING WHEAT STRAW
For incubation periods for figures on the right, see table 2

There was pronounced increase in ash content as decomposition progressed. The ash content of the aerobic material tripled and that of the anaerobic material doubled in 5 months (table 2).

The graph on vitamin content indicates the course of the changes. If the vitamins had decomposed at the same rate as the total organic matter, there would have been no increase or decrease from the initial values. An increased vitamin content indicates either a rate of synthesis exceeding decomposition or a slower rate of decomposition than that of the total organic matter. Decrease in vitamin content indicates more rapid decomposition of the vitamin than of the total organic matter. The extent of the increases and decreases are more evident

from the percentage changes shown in the lower graph on the right side of the figure.

The data on changes in vitamin content show that the aerobic composts consistently contained larger amounts of the three vitamins than the anaerobic composts. There appears to have been a trend toward first an initial increase in vitamin content of the residual organic matter and a decrease in the later periods, but this is not so apparent in the straw as in the grass or in the straw-grass mixture. The anaerobic materials showed erratic behavior: whereas there was little change in the riboflavin and the nicotinic acid, there was a pronounced decrease in the pantothenic acid; peculiarly, there was not a consistent course of change in any of the three vitamins in the anaerobic composts. The explanation for the immediate almost complete loss of pantothenic acid in the anaerobic straw is not known.

Turning now to the percentage gains and losses, one sees at once that there were large increases in riboflavin and nicotinic acid in the aerobic straw. The increase in nicotinic acid was actually greater than the increase in riboflavin, but on the percentage basis the increase in riboflavin was greater. In the anaerobic straw there were variable but small changes in riboflavin and appreciable losses in pantothenic acid and nicotinic acid. Even though decomposition of the total organic matter was more rapid under aerobic conditions, loss of the vitamins was less rapid under aerobic than under anaerobic conditions. It is apparent that decomposition of the organic matter as a whole and transformation of the vitamins followed dissimilar courses. Whereas there was progressive loss of total organic matter, at first rapid and then slow, the amounts of riboflavin and nicotinic acid actually increased rapidly at first and then decreased as rapidly until there were net losses in all cases at the end of the composting period. In all but one case the vitamin content of the composted material at the end of the experiment (167 days) was somewhat greater than it was initially, but actually vitamin loss had occurred in all six cases even though the initial content of the straw was very low.

No evidence was obtained that the straw composts ever became strongly alkaline or acid. Thus it is unlikely that any of the changes in the vitamins were the direct effects of acids or alkalies. Nevertheless considerable increases and decreases in vitamin content occurred, and it seems likely that they resulted from the synthetic and destructive activities of microorganisms.

The results obtained from decomposing grass are included in table 3 and figure 2. This was young grass that had grown rapidly and was very succulent when harvested. It was dried and ground and stored for several weeks before being used. The results show that it decomposed with remarkable speed. Under aerobic conditions about 25 per cent had decomposed in 1 week and nearly 60 per cent in a month. There was little change after a month and a half. The anaerobic material also decomposed rapidly but at a slower rate than the aerobic grass. At the last period, 146 days, both materials showed 75 per cent decomposition.

As shown by table 3 and figure 2 the initial vitamin content of the grass was

high in comparison with the straw and there was considerably more nicotinic acid than riboflavin or pantothenic acid. Even though the grass contained large amounts of the vitamins, both an apparent and a real increase occurred in all three vitamins under both aerobic and anaerobic conditions in the early stages of decomposition. Although the increases were small in most cases, between 15 and 50 per cent, the pantothenic acid content of the aerobic grass showed a gain of as much as 197 per cent at the 6-day period. The course of the vitamin changes of the grass composts were much more nearly similar than were those of the previously discussed straw composts. It is of interest that the changes were similar
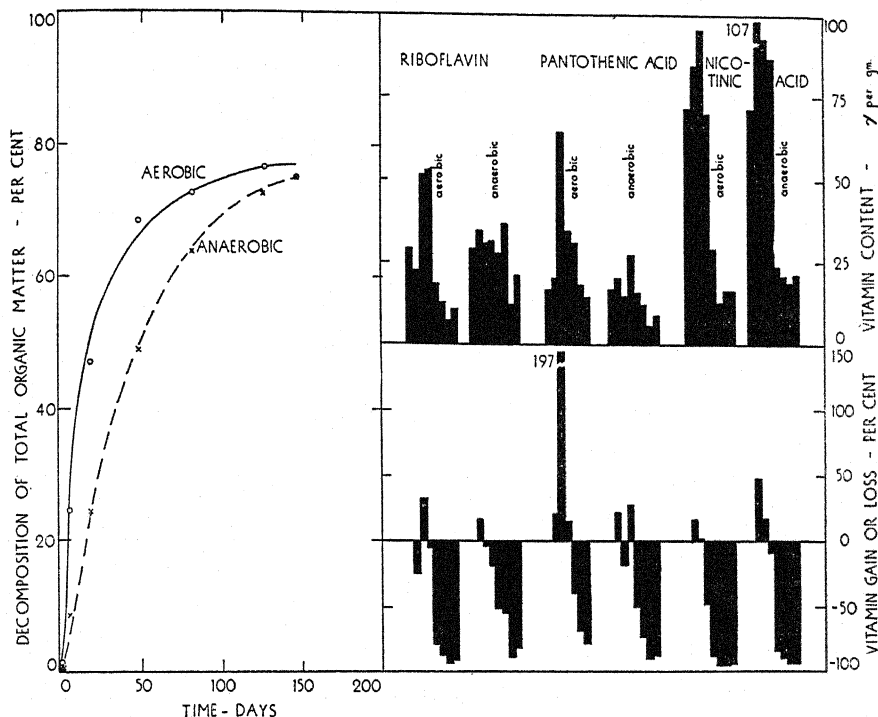

FIG. 2. CHANGES IN THE VITAMIN CONTENT OF DECOMPOSING GRASS
For incubation periods for figures on the right, see table 3

under both aerobic and anaerobic conditions. Whereas vitamin synthesis exceeded vitamin decomposition during the first 1 to 3 weeks, the opposite was true when the decomposition period was prolonged. In most cases there was either vitamin increase or slower rate of vitamin loss than decomposition of the total organic matter in the early stages of decomposition, whereas subsequently there was greater loss of vitamins than of total organic matter. Consequently, at the end of the incubation period, all of the residues had lower amounts of the three vitamins than they had initially; the loss during 146 days of composting was between 78 and 90 per cent, whereas the loss of total organic matter was 75 per cent. This is in sharp contrast to the straw, which had somewhat greater

vitamin content in the final composts than in the initial material. It seems probable that the difference is associated with the differences in initial vitamin contents of the materials. Where the initial content was low, it may have been insufficient to satisfy the vitamin requirements of the microbial cells of which the synthesis during growth kept pace with decomposition. Where the initial vitamin content was high, it should have been in excess of the amounts required for cell development, and the unassimilated portion, which would otherwise have

TABLE 3

Changes in vitamin content of decomposing grass

TIME	ASH CONTENT, DRY BASIS	RIBOFLAVIN*			PANTOTHENIC ACID*			NICOTINIC ACID*		
		From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition	From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition	From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition
days	*per cent*	γ	γ	γ	γ	γ	γ	γ	γ	γ
					Aerobic					
0	12.8	29.2	29.2	0	16.3	16.3	0	72.0	72.0	0
1	13.4	22.2	29.5	−7.3	20.0	16.5	3.5	84.7	72.8	11.9
6	17.7	51.5	38.6	12.9	64.2	21.6	42.6	96.0	95.1	0.9
19	26.7	52.5	55.1	−2.6	34.6	30.1	4.5	70.2	135.8	−65.6
48	32.5	18.9	92.1	−73.2	31.0	51.5	−20.5	29.3	227.1	−197.8
81	35.4	12.6	106.2	−93.6	18.1	59.3	−41.2	12.8	261.8	−249.0
126	35.9	7.6	125.3	−117.7	....	....	.....	16.5	308.9	−292.4
146	35.8	10.4	116.8	−106.4	14.2	65.2	−51.0	16.4	278.9	−271.5
					Anaerobic					
0	12.8	29.2	29.2	0	16.3	16.3	0	72.0	72.0	0
1	13.1	34.3	29.4	4.9	20.0	16.4	3.6	107.6	72.4	35.2
6	13.4	30.8	31.9	−1.1	14.4	17.8	−3.4	93.1	78.6	14.5
19	16.4	31.1	38.5	−7.4	27.5	21.5	6.0	87.0	95.0	−8.0
48	22.7	27.9	57.3	−29.4	15.9	32.0	−16.1	23.9	141.1	−117.2
81	27.8	36.7	80.9	−44.2	12.1	45.2	−33.1	21.4	199.4	−178.0
126	32.2	12.2	106.2	−94.0	5.7	59.3	−53.6	18.7	261.8	−243.1
146	34.5	21.3	116.8	−95.5	8.5	65.2	−56.7	21.5	287.9	−266.4

* Per gram of dry and ash-free material.

† Calculated from loss of total organic matter during decomposition.

been large at the periods of advanced decomposition, was destroyed by the microorganisms. If this was the case, the results indicate that unassimilated vitamins are readily decomposed by some members of the mixed population and that the vitamin material that was recovered from the composting materials was largely that contained in the microbial cells.

The aerobic and anaerobic materials showed no consistent differences in changes in vitamin content. Though the loss of pantothenic acid was somewhat more rapid under anaerobic than under aerobic conditions, decomposition of

nicotinic acid and riboflavin was less rapid in the anaerobic grass at most of the test periods.

The straw-grass mixtures decomposed at rates intermediate between those of the two previously discussed materials. Decomposition was rapid during the first 40 days and then became slower (table 4 and figure 3).

TABLE 4

Changes in vitamin content of a decomposing straw-grass mixture

TIME	ASH CONTENT, DRY BASIS	RIBOFLAVIN*			PANTOTHENIC ACID*			NICOTINIC ACID*		
		From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition	From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition	From assay	Calculated, assuming no loss or gain during decomposition†	Gain or loss(−) during decomposition
days	per cent	γ	γ	γ	γ	γ	γ	γ	γ	γ
				Aerobic						
0	8.4	14.9	14.9	0	9.1	9.1	0	38.8	38.8	0
1	8.6	16.3	14.9	1.4	11.5	9.1	2.4	57.6	38.8	18.8
7	10.8	32.1	19.2	12.9	53.2	11.6	41.6	64.0	49.8	14.2
20	13.8	11.0	25.0	−14.0	18.9	15.3	3.6	20.8	65.0	−44.2
40	18.2	....	....	......	....	....	......	44.4	95.8	−51.4
54	18.9	12.1	38.8	−26.7	6.2	23.5	−17.3	22.1	100.8	−78.7
75	23.0	12.2	49.6	−37.4	8.5	30.1	−21.6	35.0	128.9	−93.9
130	26.0	12.6	59.5	−46.9	7.1	36.1	−29.0	41.4	154.5	−113.1
145	27.6	11.2	63.3	−52.1	6.9	38.4	−31.5	34.9	164.4	−129.5
				Anaerobic						
0	8.4	14.9	14.9	0	9.1	9.1	0	38.8	38.8	0
1	8.5	12.2	14.9	−2.7	9.2	9.1	0.1	53.3	38.8	14.5
7	8.8	12.2	15.4	−3.2	11.9	9.3	2.6	46.2	40.0	6.2
20	10.7	12.0	18.2	−6.2	15.7	11.0	4.7	34.6	47.3	−12.7
40	12.9	....	....	......	....	....	......	19.7	60.2	−40.5
54	12.8	17.2	23.4	−6.2	8.9	14.2	−5.3	14.3	60.8	−46.5
75	14.2	17.9	26.6	−8.7	3.9	16.1	−12.2	6.8	69.0	−62.2
130	19.1	9.2	40.0	−30.8	1.9	24.3	−22.4	13.4	104.0	−90.6
145	19.3	7.9	34.2	−26.3	2.1	20.8	−18.7	10.3	89.0	−78.7

* Per gram of dry and ash-free material.

† Calculated from loss of total organic matter during decomposition.

The changes in vitamin content of the straw-grass materials were similar to those of the grass. The vitamin content of all of these composts was greater at some stages of decomposition than at the start, and during the first few days there were actual increases in the total vitamin content, with the exception of riboflavin in the anaerobic compost. After the initial gain, a loss occurred, and in most cases the decrease was greater than 50 per cent after the first 2 months. Increases in vitamin content (micrograms per gram) were relatively small after the first week, indicating that the vitamins decomposed more rapidly than the

total organic matter. All of the results from the straw-grass and grass materials are nearly enough alike to justify the following generalization: *When mixed microbial populations attack readily decomposable plant materials containing moderate to large amounts of certain B-vitamins there is at first an increase or a small loss of the vitamins and this is followed by rapid loss leading to the formation of organic residues with vitamin content lower than that of the initial plant materials.* This generalization does not apply to straw, which had a low initial vitamin content. The effects of microorganisms on other vitamins than the three studied is un-
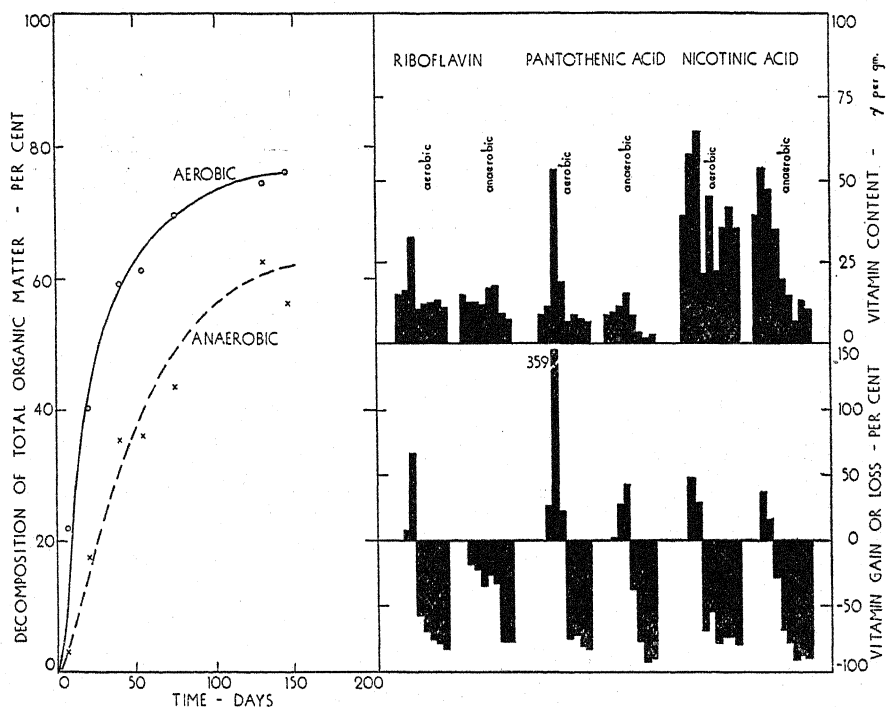
FIG. 3. CHANGES IN THE VITAMIN CONTENT OF A DECOMPOSING MIXTURE OF WHEAT STRAW AND GRASS

For incubation periods for figures on the right, see table 4

known, but it is possible that many of the vitamins of the B-complex would show similar changes since they are required for development of microorganisms and are assimilated, synthesized, and excreted to different degrees by these organisms. It may be expected that vitamins such as carotene and ascorbic acid, which seem not to be required by microorganisms, would show different changes. With such materials it is possible that decomposition is progressive, the rate of change being determined by the susceptibility of the vitamins to decomposition and to alteration by environmental effects.

The relative vitamin changes in the composts are indicated in table 5. In only one of six cases did the vitamin content of straw decrease, whereas an ulti-

mate decrease due to decomposition occurred in all cases with the grass and straw-grass composts. This indicates that the vitamin contents of various organic materials tend to become more nearly alike as decomposition proceeds. In other words, the vitamin content of vitamin-rich materials tends to decrease and that of vitamin-poor materials tends to increase with prolonged decomposition. It is apparent, however, from the results discussed above that the relationships were quite different at the early periods of composting.

It has been intimated in the preceding discussion that there were changes in reaction during composting. This suggests the possibility that reaction may have had a direct effect on the vitamins, since some vitamins are destroyed by alkaline solutions. The reaction changes in the composts are shown in table 6.

TABLE 5

Relative amounts of vitamins in organic materials before and after decomposition

(The vitamin content of the plant material containing initially the greatest amount of the vitamin is taken as 100)

ORGANIC MATERIAL	RIBOFLAVIN		PANTOTHENIC ACID		NICOTINIC ACID	
	Before	After	Before	After	Before	After
Aerobic						
Straw........................	7	11	15	31	12	26
Grass........................	100	36	100	87	100	23
Straw-grass..................	51	38	55	42	54	49
Anaerobic						
Straw........................	7	11	15	7*	12	16
Grass........................	100	73	100	52	100	30
Straw-grass..................	51	27	55	13	54	14

* This is the one exception where the vitamin content of the straw was lower at the end of the composting period than initially. In all cases the vitamin content of the grass and grass-straw composts was lower after composting.

These data show that the straw was not strongly alkaline at any time, but the aerobic grass and straw-grass materials had reactions between pH 8.0 and 9.0 at most of the test periods. These reactions were high enough to liberate free ammonia, and the materials had a strong ammoniacal odor during the first few weeks. It is possible that the reaction was responsible in part for the loss of riboflavin and pantothenic acid but it seems unlikely that it was the dominant factor for the following reasons: The reactions of the grass and straw-grass composts were strongly alkaline at the early stages of decomposition when riboflavin and pantothenic acid increased. These two vitamins passed through a similar course of change under both aerobic and anaerobic conditions, but the reactions of the anaerobic materials were not strongly alkaline at any of the test periods. Changes in nicotinic acid were much the same as those for the other two vitamins, but nicotinic acid is not sensitive to alkalinity, whereas both riboflavin and pantothenic acid are broken down by strong alkalies. Proof is lacking that similar

factors were concerned with the changes of the three vitamins, but the similarities of the data suggest that the increases and decreases were due to microbial development, and that reaction had little if any effect on these changes.

TABLE 6

Changes in reaction during decomposition of organic materials

INCUBATION	AEROBIC	ANAEROBIC
days	*pH*	*pH*
Straw		
7	6.2	5.9
20	7.1	5.9
44	7.3	6.7
72	5.7	5.9
127	6.2	6.8
153	6.0	7.0
167	6.0	7.2
Grass		
1	8.2	5.7
6	8.6	5.3
19	8.1	5.5
48	8.1	6.4
81	9.1	7.8
146	8.6	7.7
Straw-grass		
1	6.3	5.8
7	8.7	5.2
20	8.7	5.2
40	8.5	6.5
54	7.0	5.7
75	9.4	7.7
130	9.0	6.5
145	9.0	7.3

DISCUSSION

Limitations of the results

Numerous factors may have affected the accuracy and significance of the results. There was an error in sampling inherent in heterogeneous materials. Particular difficulty was encountered in obtaining representative samples of the wet anaerobic materials. Some of the inconsistencies, particularly those with the straw, are believed to have been caused by sampling difficulties. Of no less importance was the limitation of the extraction procedure. Material for riboflavin assays was extracted with $0.1N$ HCl at 15 pounds' steam pressure for 30 minutes. The extracts for assays of the other two vitamins were made with water at neutrality, heated as above. The cooled mixtures were adjusted to

neutrality, made to volume, and centrifuged. The supernatant liquid was used for the assays. Possibly the vitamins were not recovered with the same degree of completeness from the various composts. It is to be further emphasized that these studies were made on very complex mixtures of organic materials which were being continuously changed by decomposition. Possibly materials were present that activated, inactivated, or substituted in part for the vitamins, thus giving results that did not truly indicate the vitamin content of the material being analyzed. It is not possible to assess the effects of these factors, but it is not believed that they appreciably affected the trends of the results.

It is tempting to generalize about other vitamins of the B-complex on the basis of the results on riboflavin, pantothenic acid, and nicotinic acid. These are, however, only three of numerous vitamins having different chemical characteristics. There is reason to believe that they are as a whole unstable, since it is because of their chemical reactiveness that they serve as vitamins. Furthermore, they occur in the soft growing tissues of plants which are readily decomposed, and the vitamins therefore lack the protection afforded some structural components of plants by incrusting materials.

Vitamin synthesis

There is abundant evidence that vitamins are produced by microorganisms. In fact, some organisms produce vitamins in such abundance that they can serve for commercial vitamin production. Burkholder reported (9) that, under certain cultural conditions, *Candida guilliermondia* produced so much riboflavin that crystalline riboflavin accumulated in the medium. Foster (17) reported that he had an organism with the remarkable ability of producing riboflavin in amounts exceeding those of the microbial cells. As regards the synthesis of vitamins by microorganisms and the availability of the vitamins to associated organisms, the results of Thompson are particularly significant (55). He found that considerable amounts of various B-vitamins were excreted by bacteria into their substrate, and the amounts excreted frequently exceeded the amounts contained in the cells. Furthermore, vitamin synthesis was not appreciably affected when large amounts of vitamins were included in the medium.

Some evidence concerning the bacterial excretion of riboflavin has been obtained in the author's laboratory from studies with cultures of *Azotobacter*. These cultures were grown on nitrogen-free media containing 1.5 per cent dextrose. The following results were obtained with cultures which made fair to good growth during the 10 days preceding analysis for riboflavin:

CULTURE	PIGMENTATION	RIBOFLAVIN		
		Micrograms per ml., total culture	Micrograms per ml., centrifuged liquid	Per cent excreted
Uninoculated.............	....	<0.001	<0.001	..
Az. agile..................	None	0.25	0.21	84
Az. chroococcum...........	None	0.22	0.13	59
Az. vinelandii.............	Fluorescent green	0.34	0.24	71

These results are similar to those of Thompson in that they show that a large portion of the synthesized vitamin may be recovered outside the microbial cells. These cultures produced several times the amounts of riboflavin mentioned above when the medium contained ethanol in place of glucose.

Synthesis of vitamins is so common among microorganisms that there can be no doubt that vitamins exist in soils. Some of the evidence regarding the occurrence of B-vitamins in soils has been discussed in the introduction of this paper. As regards the vitamin content of soils, little is known other than that vitamins are present in soil, that they occur in greater amounts in soils well provided with organic matter than in infertile soils, and that they are concentrated near the soil surface. This is what would be expected, since the vitamins in soils are known to occur in plant residues and are produced by microorganisms that attack these residues. Whether or not the vitamins occur principally in the free or in the bound state has not been established. Furthermore, no information has yet been obtained regarding the influences of abundance and types of microorganisms in soil on vitamin content or the direct and indirect effects of higher plants through roots during periods of growth or through the organic residues after death of the plants. Interesting and significant relationships doubtless exist. The results obtained from the compost studies can serve as a basis for speculations regarding the relationships, but more specific information is required to establish the conditions that actually exist.

Absorption of vitamins by higher plants

If it is assumed that vitamins occur in soils in the free state, it is important to establish whether or not higher plants are able to absorb them. The studies on cultivation of excised plant roots are of particular interest in this connection. The results of Robbins (36), White (60, 61), Bonner (2, 4, 6), and others on cultivation of excised plant roots clearly indicate the ability of roots to absorb various organic materials including vitamins. In fact, some roots require preformed thiamine, nicotinic acid, or pyridoxine or various combinations of these vitamins and possibly other as yet unidentified accessory growth substances. Carpenter (11) obtained evidence of absorption of riboflavin by studies of the vitamin content of sap of decapitated plants. When such plants were kept in solutions containing riboflavin, the plant sap contained many times as much riboflavin as the sap from plants in vitamin-free solutions. Von Hausen (21, 22) reported that peas grown in solutions containing vitamin C made more growth and contained larger amounts of ascorbic acid than the control plants.

The fact that plants are able to absorb vitamins from soil provides no indication of the amount of absorption which occurs when the plants are grown under farming conditions, but the possibility exists that absorption occurs. Even though vitamins are absorbed from the soil, it may be that the plant profits no more from the material so derived than from that provided by the aerial part of the plant.

Microorganisms in the rhizosphere

In any consideration of the influences of soil-contained vitamins on plant development, the importance of microorganisms should not be overlooked, since both vitamin synthesis and vitamin decomposition are closely associated with microbial development. There are two principal centers of extensive microbial growth in soil, one of which is plant residues and the other the rhizosphere. It seems probable that the latter is of particular importance. It has been shown that plant roots support an abundance of microorganisms (50). As producers of vitamins in direct contact with the absorbing system of the plant, it is reasonable to expect that the rhizosphere organisms have a greater effect than the microorganisms developing at some distance from the root surfaces. The probability gains weight in the light of the results obtained from the compost studies which indicate that vitamins are readily decomposed. Unless the roots are close to the regions where the vitamins are produced, the vitamins might not persist long enough to become absorbed.

Influence of vitamins upon development of higher plants

Many attempts have been made to establish whether or not higher plants are favorably affected by an external supply of vitamins. These studies have yielded many conflicting reports and, by careful selection of the published data, one could present considerable evidence to show that development of higher plants is favored by vitamin treatment; on the other hand, by similar treatment of the data, a stronger case could be developed on the thesis that vitamin treatment has no appreciable influence on plant development. A comprehensive discussion of the subject would be out of place here, but reference to some of the results will serve to indicate the trends in development of the subject.

Attention has been directed to two different effects that vitamins might have on plant development: change in the vitamin content of plants where there are differences in the amounts of the vitamins in the external medium; changes in plant growth, such as overall development, rooting, or flowering in response to vitamin treatment.

The results of Bottomley and Mockeridge [see Schopfer (45)], which are frequently cited, led to the conclusion that development of higher plants is stimulated by organic substances of microbial origin. It was even assumed that these accessory growth substances were necessary for maximum plant development. Whereas the value of organic matter as a source of mineral plant nutrients is generally recognized, it was the opinion of Clark (12, 13, 15), Wolfe (62), and others that accessory growth substances were not required by higher plants and that organic substances had no effect when plants were grown in properly balanced mineral solutions. Viswanath (56) reported that organic manures increased yields over those obtained from mineral fertilizers and the seeds of plants treated with the manures had greater vitality and produced superior plants. McCarrison reported that the seeds of manured plants had higher vitamin content (30, 31). These unusual claims did not pass unnoticed. Some results

confirmed those of Viswanath and McCarrison. It was reported, for example, by Rowlands and Wilkinson (38) that seeds from manured soils had greater amounts of vitamins, which were believed to be derived from the soil. Hartley and Greenwood (20) found that small amounts of farmyard manure increased growth of cereal crops out of proportion to their contribution of plant nutrients. On the other hand, more convincing evidence has been presented in support of the idea that organic manures have little or no characteristic effect on the vitamin content of plants. Harris (19) and Leong (25) found no appreciable differences in the thiamine content of grains from manured and mineral-fertilized soils of the Rothamsted plots. It was noted by Clark (14) that certain plants which had been grown in absence of microorganisms for many generations had virtually the same content of vitamin A as the same kind of plants grown under nonsterile conditions. Extensive studies were carried out by Scheunert and associates on the effects of animal manures and mineral fertilizers on the vitamin content of cereal grains and vegetables. These results indicated that the type of fertilizer treatment had little or no effect on the amounts of thiamine, vitamin A, or vitamin C in the plants (39, 40, 41, 42, 43, 44).

Particular interest in the influence of thiamine on plant growth followed the publication of results by Bonner and Greene (3, 5), showing that many plants and cuttings made much better development when they were provided with an external source of the vitamin. Dennison subsequently reported that riboflavin increased growth of eggplant and that vitamin C increased growth of tobacco (16). Hitchcock and Zimmerman observed a thiamine response of asters grown in composted soil which suggested that the vitamin was beneficial under conditions of limited nutrient supply (23); when the plants were cultivated in flats there was definite response to thiamine, but no such response was observed when the plants were grown in pots. According to McBurney, Bollen, and Williams (29), pantothenic acid had a favorable effect on growth of uninoculated alfalfa, and these workers concluded that the symbiotic bacteria favor development of the legumes not alone through their relationships with the nitrogen-fixing mechanism but also by providing a source of needed pantothenic acid.

Subsequent studies have shown that the optimism generated by the early reports was unjustified. Bonner altered his earlier position and asserted that the popular notion that thiamine promotes growth of horticultural plants is untenable (7). Arnon noted no benefit from thiamine on various plants grown in culture solutions under carefully controlled conditions (1). Hamner (18) likewise observed no beneficial effects under conditions of varied nutrient supply. Hitchcock and Zimmerman failed to verify the results of others that thiamine had favorable effects on rooting of cuttings (23). Negative results were also reported for vegetables grown in sand culture and treated with thiamine, riboflavin, and pyridoxine (33). Many tests have been made with several vitamins on vegetables, grasses, flowering plants, and trees under a variety of soil conditions, and in virtually all cases there were no apparent beneficial effects. The conclusion of Siddappa and Subrahmanyan (47) that plants fail to respond to

organic materials when all the necessary minerals are available is shared by many.

Although it cannot be asserted without qualification that plants do not benefit from an external source of vitamins, most of the recent evidence supports this point of view. According to Maynard (32), genetic and climatic factors have much greater effects on the vitamin content of plants than do soil factors. More fundamental studies are needed before it will be possible properly to evaluate the importance of such soil-contained organic materials as vitamins, auxins, and related substances in development of higher plants. The possibility that vitamins and other organic substances contained in soils and originating from microbial development have important effects on higher plants has not been eliminated (8) and justifies careful study, particularly since exaggerated claims have been made of benefits to be derived from such substances. It seems apparent from recent studies, however, that the likelihood of obtaining superior plants simply as a result of adding vitamins to soils is remote.

Plant residues as sources of vitamins

In the preceding discussion evidence has been presented to show that vitamins are synthesized by microorganisms, that they occur in soils, and that they can be absorbed by higher plants. They are, however, susceptible to rapid destruction by mixed populations of microorganisms such as exist in soil, but it is likely that, at least under certain conditions, the soil provides a supply of vitamins to growing plants. Whether or not plants utilize such vitamins and profit from them has not been established.

The studies on composting organic matter suggest the nature of the changes which take place during decomposition of organic matter incorporated in soil. It is probable that considerable amounts of vitamins are synthesized, at least during early stages of decomposition, but, in view of the temporary persistence of the vitamins, a considerable portion of the substances synthesized, following incorporation of green manures, cover crops, and similar plant residues, may be dissipated by the time crops are sufficiently established to make use of these substances. Contrary to the frequently expressed opinion, well-composted material is a poorer source of certain vitamins than are fresh plant residues. Consequently, if organic matter has any value as a source of these vitamins for plant development, it should be more effective in this regard if decomposition takes place in the soil coincident with plant development.

SUMMARY

The nicotinic acid content of certain plant residues varied from 2.4 to 85 γ per gram (on the dry basis); most residues contained less than 30 γ. The nicotinic acid content was greater than that of pantothenic acid or riboflavin. The vitamin content was low in residues containing large amounts of structural tissue. Nicotinic acid decomposed in composts prepared from various plant residues and, during the latter stages of composting, the rate of loss was greater than that of the total organic matter.

Changes in the amounts of riboflavin, pantothenic acid, and nicotinic acid in composts of wheat straw, grass, and a straw-grass mixture were also determined. During the first week or two of composting an actual increase in vitamin content was generally noted accompanying the rapid initial microbial development. As decomposition of the organic matter progressed, vitamin content markedly decreased. Materials that initially had high vitamin content lost a large part of the vitamins, and the well-composted materials had lower vitamin content than the plant substances from which the composts were prepared. The percentage losses were lower with plant materials that initially contained small amounts of vitamins. The composting materials tended to become alike in vitamin content, irrespective of their initial vitamin contents.

Decomposition of the total organic matter was slower under anaerobic than under aerobic conditions, and there were not so great increases in vitamin content during early stages of decomposition and somewhat greater percentage losses after prolonged composting. The general order of the changes was similar, however, under both aerobic and anaerobic conditions. In some cases the vitamin content increased 200 to 300 per cent during the first week or two of composting.

The importance of vitamins in plant development and the relationships of microorganisms to the vitamin content of soils was discussed.

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EFFECT OF MEDIA COMPOSITION ON THE NUMBERS OF BACTERIAL AND FUNGAL COLONIES DEVELOPING IN PETRI PLATES[1]

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In a recent study to determine the relative number of colonies of fungi and bacteria isolated from different composts of soil and crop debris, the difficulties usually encountered in Petri plate technique prevented accurate results being obtained. The main difficulty was that one or more of the fast growing colonies of fungi often covered the plates within 3 days, masking the more slowly growing colonies, and too often completely covering them. Similarly in the case of the bacterial dilutions, the presence of fast growing or spreading types of colonies made an accurate count impossible. In view of the situation, it was decided to investigate the effect of certain media and chemicals which might be fairly selective and at the same time control the growth-rate of both fungal and bacterial colonies without inhibiting the slow-growing ones. Some of the data obtained are presented briefly.

MATERIALS AND METHODS

Eighty chemicals were tested, and from preliminary observations of these the treatments showing the most promise were selected for further study. The list of chemicals was finally narrowed to lithium oxide and boric acid. These two chemicals were added separately to each of two types of media in various concentrations as specified in table 1.

The potato-dextrose-agar medium was made up in the proportions of 300–30–15 gm. per liter. The formula by Lipman and Brown[3] was used for the synthetic medium as follows: dextrose 10 gm., K_2HPO_4 0.5 gm., $MgSO_4 \cdot 7H_2O$ 0.2 gm., peptone (Bacto) 0.05 gm., agar 15(20) gm., distilled water to 1 liter.

With the exception of sulfuric acid in treatments 12 and 24, which was added just before the plates were poured, the various additions were made to the basic media before sterilization. The soil compost dilutions were 1:20,000 for fungi, and 1:500,000 for bacteria. One milliliter per plate was added to sets of seven replicate plates for each treatment. Counts were made of bacteria and actinomycetes or fungi 2 days after plating and on the third, fourth, fifth, and seventh days on any plates where counts could still be made. The data obtained are given in table 1.

TABLE 1

Relative effect of lithium oxide and boric acid on growth of bacteria, actinomycetes, and fungi in Petri plate culture

NO.*	MEDIUM†	CHEMICALS ADDED PER LITER		DAYS AFTER PLATING					REMARKS
		Chemical	gm.	2	3	4	5	7	
				Bacteria and Actinomycetes‡					
1	Syn.	.....		57	77	88	136	165	Large bacterial colonies did not develop
2	Syn.	LiO₂	0.07	50	65	77	113	164	
3	Syn.	LiO₂	0.10	34	48	68	99	118	
4	Syn.	LiO₂	0.14	19	48	59	86	106	
5	Syn.	LiO₂	0.18	15	40	67	87	90	
6	P.D.A.	.....		75	62				Large bacterial colonies covered smaller ones after second day
7	P.D.A.	LiO₂	0.07	73	56				
8	P.D.A.	LiO₂	0.10	69	44				
9	P.D.A.	LiO₂	0.14	74	57				
10	P.D.A.	LiO₂	0.18	76	59				
				Fungi§					
11	Syn.	....		18	646				
12	Syn.	0.5 N H₂SO₄	13.0	14	52				
13	Syn.	H₃BO₃	0.9	52	722				
14	Syn.	H₃BO₃	1.2	8	722				
15	Syn.	H₃BO₃	1.5	12	700				
16	Syn.	H₃BO₃	1.8	2	652				
17	Syn.	H₃BO₃	2.1	6	698				
18	Syn.	H₃BO₃	2.4	8	648				No bacterial colonies
19	Syn.	H₃BO₃	3.0	0	712				
20	Syn.	H₃BO₃	3.6	0	646				
21	Syn.	H₃BO₃	4.2	0	516				
22	Syn.	H₃BO₃	6.0	0	268				
23	P.D.A.	....		22					Spreading colonies of bacteria
24	P.D.A.	0.5 N H₂SO₄	13.0	80	72				
25	P.D.A.	H₃BO₃	0.9	78	152				
26	P.D.A.	H₃BO₃	1.2	40	332				
27	P.D.A.	H₃BO₃	1.5	42	208				Large fungal colonies covered small ones
28	P.D.A.	H₃BO₃	1.8	22	260				
29	P.D.A.	H₃BO₃	2.1	52	334				
30	P.D.A.	H₃BO₃	2.4	52	398				
31	P.D.A.	H₃BO₃	3.0	20	574				
32	P.D.A.	H₃BO₃	3.6	6	466				Fungal colonies all small
33	P.D.A.	H₃BO₂	4.2	4	580				
34	P.D.A.	H₃BO₃	6.0	0	486				

* Treatment.

† See text for formulas used for synthetic (Syn.) and potato-dextrose-agar (P.D.A.) media.

‡ Bacteria and actinomycetes, in millions per gram.

§ Fungi, in thousands per gram.

RESULTS

The synthetic medium was very satisfactory for counts of bacteria and actinomycetes, because of the small size of the colonies and the clarity of the medium. It was possible to make counts long past the seventh day, although none were recorded.

The potato dextrose agar was unsatisfactory in comparison with the synthetic medium. The colonies increased in size so rapidly that after the second day many of the smaller ones were overrun and obscured. This decrease in observed numbers between the second and third days may be noted in treatments 6 to 10.

Lithium oxide did not prove satisfactory, since it reduced not only the number of colonies of bacteria, but those of fungi. Other chemicals found useful in suppressing fungal growth were chromic acid and sodium molybdate, but these also inhibited the growth of bacteria. They may be of some value, however, in media intended for the elimination of certain species of bacteria.

According to the results obtained, the effect of boric acid in suppressing the growth of bacteria and actinomycetes was marked in both kinds of media. For suppressing bacterial growth completely and at the same time permitting the appearance of the maximum number of fungal colonies, a concentration of 3 gm. per liter seemed most satisfactory. Boric acid, when added to potato dextrose agar (already too rich in nutrient), entirely eliminated the growth of the fast-spreading types of bacterial colonies.

The addition of sulfuric acid to either medium inhibited bacterial growth but also suppressed the growth of fungi to a point where the data were not acceptable. The data in treatments 12 and 24 indicate that the number of colonies of fungi appearing on the plates treated with mineral acid were but a small fraction of the spores actually present. The pH of the medium with H_2SO_4 added was 4.6.

No bacterial colonies were present in treatment 16, and only 2 or 3 per plate occurred in treatment 15. In the potato-dextrose-agar series, bacteria were wholly suppressed at the concentration of boric acid used in treatment 29. Some fungi grew too actively in treatments 25–30; colonies commonly attained diameters exceeding 35 mm. after 2 days' incubation and covered many of the more slowly growing species (which constituted the majority in all cases). The pH values of the media in treatments 22 and 34, which received the heaviest application of boric acid, were 6.0 and 5.8, respectively. Since these values are well within the limits for bacterial growth, the inhibition observed is a chemical effect and not one of hydrogen-ion concentration.

The synthetic medium was also found to be the most satisfactory from the standpoint of obtaining the total number of fungal colonies. Because of the restricted type of growth of the colonies, however, they were not distinguishable as to genus. On the other hand, the genus of most of the colonies on the potato dextrose agar could be recognized by the fifth day or soon after. The difference in growth of fungi on the two media was best shown at the higher concentrations of boric acid.

SUMMARY

From the results of this study it is evident that the addition of boric acid to either potato dextrose agar or the synthetic media used, effectively suppressed

the growth of bacteria but permitted a satisfactory count of fungi. Moreover, since boric acid suppresses bacterial growth by means other than an increase of the hydrogen-ion concentration, it may be added to the medium before it is sterilized, instead of when the plates are poured. Finally, a higher count of fungi was obtained in the presence of boric acid than sulfuric acid.

INFLUENCE OF CROPPING, RAINFALL, AND WATER TABLE ON NITRATES IN EVERGLADES PEAT

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The total nitrogen content of Everglades peat ranges from 3.0 to 3.8 per cent in the more weathered surface horizons and is somewhat less at lower depths. Almost all of the vast area of this peat is underlain with limestone rock, and this makes it unnecessary to add lime in the management of this soil for cropping. From the standpoint of reaction, therefore, the peat and muck soils of the Everglades are always in an optimum condition for nitrification.

Fertilizer experiments on Everglades peat have shown that grasses do not respond to nitrogen (5) except for brief periods following heavy rains. There is the same lack of response from cultivated crops except a few that are very heavy feeders of plant nutrients. Beckenbach (2) reports benefit from nitrogen fertilizer for celery grown on this type of peat. Crop requirements for nitrogen in organic soils in other regions may differ from those of the Everglades. Thus Alway (1) says that low-lime peats in Minnesota are more likely to need nitrogen in the fertilizer program than high-lime peats. Harmer (3) mentions a similar condition for Michigan peats and points out the ill effects of the presence of too much available nitrogen, especially if it is not rightly balanced with other plant nutrients.

Field and greenhouse experiments on the availability to crops of the nitrogen of Everglades peat have been reported (4) in which nine successive greenhouse crops removed 10.98 per cent of the total nitrogen of the peat. These crops were fertilized with all necessary plant-food elements except nitrogen. Dallis grass was grown on field plots of the same type of peat fertilized in a similar manner (5). During a period of 40 months 9.16 per cent of the total nitrogen of the peat, calculated to a depth of 1 foot, was contained in cuttings removed from the plots.

Even though there is not much need of nitrogen in the present fertilizer program on this peat, it is possible that added nitrogen might be beneficial after these soils have been cropped for a longer time. Hence it was thought advisable to ascertain how much nitrification is taking place in Everglades peat and to obtain some information on the effects of cropping, rainfall, and water control on the production and accumulation of nitrates. The possible loss by leaching is also being studied (6) and will be reported later.

The data reported here indicate that rapid nitrification occurs in Everglades peat and much of this takes place in the surface layers (8). Rainfall may be expected to carry nitrates downward, loss by leaching occurring during the rainy season of the year, which is normally from June to October.

EXPERIMENTAL

In October, 1934, nitrate nitrogen was determined in composited samples at different depths in a Dallis grass pasture which had been fertilized for 2 years

with an 0–12–24 mixture. At the same time samples were taken in a similar manner from an area kept in clean fallow adjacent to the pasture. The water table for both of these areas is held at the 22- to 24-inch level by means of pumps. Nitrates were determined by the Devarda reduction and distillation method using clarified water extracts of fresh undried samples (7, pp. 350–352). In table 1

TABLE 1

Nitrate nitrogen accumulation and moisture in Everglades peat at different depths and seasons on cropped and uncropped land

LOCATION	DEPTH	OCT. 17, 1934		APR. 4, 1935		JULY 22, 1935		MAR. 31, 1936	
		NO₃ nitrogen	Moisture	NO₃ nitrogen	Moisture	NO₃ nitrogen	Moisture	NO₃ nitrogen	Moisture
	inches	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
Dallis grass pasture	0–6	29	66.3	239	59.5	27	64.1	31	61.5
	7–12	37	74.8	336	71.1	154	74.6	51	75.3
	13–18	31	80.2	166	78.7	111	71.8	32	79.9
	19–24	36	80.6	122	78.2	111	78.9	32	79.1
Fallow land next to pasture	0–6	31	62.0	410	62.7	66	61.8	260	58.8
	7–12	64	67.8	422	70.3	245	72.9	278	73.5
	13–18	102	78.0	412	77.8	301	78.5	236	80.1
	19–24	6	76.7	171	77.8	299	79.7	216	80.6

TABLE 2

Rainfall preceding and during period covered by table 1

MONTH	1934	1935	1936	AVERAGE 1924–1942
	inches	*inches*	*inches*	*inches*
January	0.14	0.30	1.91	1.83
February	1.91	1.32	4.04	1.92
March	7.10	0.41	2.40	3.31
April	3.11	5.32	1.96	3.66
May	5.20	1.08	6.39	4.48
June	10.15	8.45	18.61	10.61
July	10.09	6.35	6.09	7.35
August	12.41	6.54	5.33	7.85
September	7.44	10.88	5.84	8.82
October	3.22	5.71	1.65	4.47
November	0.65	0.36	9.17	2.36
December	0.82	2.07	1.18	1.42

and in subsequent tables they are reported as parts per million on the moisture-free basis, whereas the soil moisture is given on the moist-soil basis.

As recorded in table 1 nitrates were low from the surface downward in both the pastured and the uncropped areas. The rainfall record of table 2 for the months preceding October indicate that considerable leaching had taken place. During the next few months very little rain fell (table 2) and there was no leaching. By April 4, 1935 the accumulation of nitrates was several times greater than it

was in October, particularly in the surface 18 inches of the fallow area (table 1). Nitrate accumulation was not so great in the pasture because of the growth of the grass. It may be observed that the amount of moisture in the peat increased from the surface downward to a depth of 18 inches and in April the surface 6 inches in the pasture was somewhat drier than in the fallow land.

In these two areas nitrates were determined for the same depths in July, 1935 and in March, 1936 (table 1). Enough rain fell in June and July, 1935 (table 2) to cause an appreciable leaching of the nitrates that accumulated during the previous dry months. Considerable rain fell in September and October, 1935 as well as in February, 1936, and the accumulation of nitrates (March 31) was not so great in the fallow land as it was on April 4 of the previous year. Because of this smaller accumulation the pasture grass caused nitrates to be especially low by March 31, 1936, and the grass would probably have responded at that time to a nitrogenous fertilizer.

TABLE 3

Nitrate nitrogen and moisture content of cropped and fallow areas of fields with different water-table levels, January 7, 1936

DEPTH OF WATER TABLE	DEPTH OF SAMPLE	AREA IN SUGAR CANE		AREA IN FALLOW	
		NO$_3$ nitrogen	Moisture	NO$_3$ nitrogen	Moisture
inches	inches	p.p.m.	per cent	p.p.m.	per cent
12	0–6	150	64.10	141	66.60
	7–12	205	72.63	154	76.78
24	0–6	160	60.03	149	57.93
	7–12	187	67.71	205	67.80
36	0–6	167	60.38	136	60.98
	7–12	253	67.62	202	70.69

Another comparison of nitrates in cropped land and in clean fallow was made in three fields in each of which the water-table level was held at different depths (table 3). For the fields with 12-inch water tables it may be noted that there was no marked difference due to cropping for either the 0- to 6- or 7- to 12-inch depths. The data show that a similar lack of difference existed in the peat on the fields with 24- and 36-inch water tables. Accumulation of nitrates under the growing sugar cane would probably have been less were it not for the plant residue that is left on the land, as the cane stems only are removed in the harvest process.

The rainfall preceding the sampling of these fields is given in table 2. No rain fell in January, 1936 up to the time of sampling. The 2.07 inches of rain in December, 1935 probably caused some of the nitrate to leach out of the 0- to 6-inch zone, as the accumulation is greater in the 7- to 12-inch layer for all water-table depths, including the field where it is held at 12 inches below the surface. The saturated condition of the peat in the 7- to 12-inch level in this field precludes the possibility that nitrates are found to any extent in that zone.

The fields in clean fallow with 12- and 24-inch water tables were sampled again

on March 31, 1936 (table 4) and to a greater depth.   In the field with the 24-inch water table the accumulation of nitrates had increased, and the concentration was almost as high in the 13- to 18- and 19- to 24-inch levels as in those above. As indicated in table 4, the peat below a depth of 12 inches was saturated with water on both fields.   A total of 8.35 inches of rain in January, February, and March (table 2) probably accounts for the deep penetration of nitrates.

Nitrates were determined on May 22, 1936 in the surface 6-inch layer of Everglades peat on which corn was growing in 3-foot rows with hills 2 feet apart in the row.   Grass and legume cover crops as well as the manure (table 5) had been

TABLE 4

Nitrate nitrogen and moisture content of Everglades peat at different depths in fallow areas of fields with 12-inch and 24-inch water tables, March 31, 1936

DEPTH OF WATER TABLE	DEPTH OF SAMPLE	NO₃ NITROGEN	SOIL MOISTURE	DEPTH OF WATER TABLE	DEPTH OF SAMPLE	NO₃ NITROGEN	SOIL MOISTURE
inches	*inches*	*p.p.m.*	*per cent*	*inches*	*inches*	*p.p.m.*	*per cent*
12	0–6	145	63.4	24	0–6	260	58.8
	7–12	173	75.0		7–12	278	73.5
	13–18	136	80.2		13–18	236	80.1
	19–24	65	81.4		19–24	216	80.1

TABLE 5

Nitrate nitrogen in the surface 6 inches of corn cover crop plots, May 22, 1936

PLOT NUMBER	NO₃ NITROGEN			
	Plot under legume cover crop	Plot under grass cover crop	Plot in clean fallow	Manured plot
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	376	455	323	...
2	305	403	283	263
3	345	252	299	389
4	332	323	254	317
Average.......	340	358	290	329

turned under several weeks previous to the planting of the corn in early March. The fallow plots had been kept clear of growth from the time of the corn crop of the previous year.   There was a good stand of corn in the soft dough stage at the time the field was sampled for nitrates in May.   Table 5 shows that nitrates were not significantly lower in the plots previously fallowed than in the other plots. All of the plots had an ample supply of nitrate nitrogen, possibly too much for a well-balanced plant-nutrient condition, despite the fact that no nitrogen was used in the fertilizer for the corn.   As recorded in table 2 there were moderate amounts of rain during the period preceding the soil sampling and only 2.13 inches fell in May up to May 22.   It is unlikely therefore that much nitrate had been leached out of the surface 6 inches.

Nitrates were also studied for this same period in another vigorously growing field of corn, and as shown in table 6, the amounts present were ample for the needs of the crop without the addition of any in the fertilizer. Nitrate accumulation in a noncropped area adjacent to the corn field gave an indication of how much nitrogen the growing corn had utilized. It may be noted that nitrates were lower in the second 6-inch layer than in the surface and were similar in both the cropped and uncropped areas.

On April 8, 1936, nitrates were determined in a field of celery at the time of harvest. As shown in table 7 the amounts present were low, irrespective of

TABLE 6

Effect of a vigorously growing crop of corn on the nitrate content of Everglades peat

DEPTH OF SAMPLING	NO₃ NITROGEN	
	Clean fallow	Corn growing
inches	*p.p.m.*	*p.p.m.*
0–6	239	145
7–12	127	130

TABLE 7

Nitrate nitrogen in Everglades peat at time of harvest of a crop of celery and 6 weeks later

CROP	DEPTH OF SAMPLING	WITH SUMMER FALLOW		WITH SUMMER GREEN MANURE CROPS	
		NO₃ nitrogen	Soil moisture	NO₃ nitrogen	Soil moisture
	inches	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
Celery at time of harvest	0–6	33	53.0	36	52.0
	7–12	19	70.1	27	65.6
	13–18	46	75.7	76	75.6
	19–24	43	78.3	18	79.8
6 weeks after celery harvest	0–6	160	n.d.*	172	n.d.
	7–12	153	n.d.	186	n.d.
	13–18	128	n.d.	210	n.d.
	19–24	124	n.d.	180	n.d.

* n.d. = not determined.

whether the land had been planted to a green manure crop or kept fallow. Table 2 records the rainfall during the months preceding April, and none fell during the first 8 days of April. The high transpirational requirements of the celery as indicated by the moisture content of the peat (table 7) precludes the possibility that any nitrate was leached out of the root zone, and the small amount of nitrate present on April 8 indicates that the celery would have responded to a nitrogenous fertilizer. Fertilizer experiments as reported by Beckenbach (2) corroborate this.

Succulent material such as the roots and trimmings of celery plants may be expected to decompose rapidly in the presence of sufficient warmth and moisture.

The discarded stems of the celery in the aforementioned plots were turned under at the time of harvest on April 8, and soil samples were taken 6 weeks later for nitrate determinations. As recorded in table 7, a considerable accumulation had taken place in that comparatively short period. The 1.81 inches of rain that fell April 25 and 26 probably accounts for the rather deep penetration of nitrates.

SUMMARY

From October, 1934 to June, 1936 the accumulations of nitrate nitrogen were studied in various cropped and uncropped areas of Everglades peat. The desired soil water levels in these fields are kept fairly constant by means of pumps. In this nitrate accumulation study the fertilizers used for the crops contained no nitrogen.

Conversion of the nitrogenous material of the peat was rapid and there was no indication that the pasture grass, corn, and sugar cane would have responded to a nitrogenous fertilizer except for brief periods following a succession of heavy rains.

Nitrate accumulation was low under a crop of celery irrespective of whether the land had been in clean fallow or a cover crop had been turned under. Nitrates were not significantly higher under corn where a cover crop had been used.

Rainfall carried nitrates downward, and at the end of the rainy season the accumulations of nitrates were very considerably reduced. During the dry season, nitrates were ample for crops of moderate nitrogen requirements even where the water table was held as high as 12 inches below the surface of the peat.

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LEAF COMPOSITION IN RELATION TO THE MINERAL NUTRITION OF TUNG TREES

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Lear or foliar analysis[2] has become well established in recent years as a useful aid in determining the nutrient requirements of a wide variety of crops. Lagatu and Maume (16) in the last two decades laid the groundwork for the systematic use of leaf analysis in studying the absorption of mineral elements as affected by various growth factors. Their findings have been extended by Thomas (25), who has contributed much in this field. That considerable emphasis is now being placed on leaf analyses is readily seen from the large volume of literature that has accumulated on this subject in the last few years.[3]

Leaf analysis has become especially useful as an aid in studying the mineral nutrition and diagnosing the fertilizer requirements of tree crops (1, 3, 5, 6, 11, 12, 20, 23, 27). Most of this work has been concerned with a limited number of chemical elements, and frequently the analysis has been for only one element. It has become increasingly apparent from recent investigations that the ratios and interactions of certain elements are as important as the total amounts present (4, 8, 9, 24). This would indicate the desirability of making rather complete leaf analyses to ascertain more acurately the nutritional status of the plants under investigation.

As the tung tree is comparatively new to the United States, it is thought that leaf analysis, correlated with soil conditions, fertilizer treatment, and tree performance, will prove very helpful in determining its mineral nutrition and fertilizer requirements.

SCOPE OF INVESTIGATION

Several investigators (5, 15, 23, 26) have shown that the composition of leaves of a plant varies with the age of the leaf, with soil conditions, and with fertilizer treatment. As a preliminary step it appeared desirable, therefore, in the use of leaf analyses of tung for diagnostic purposes, to obtain information on the mineral composition of the leaf as influenced by these various factors under field conditions. Analyses were made for nitrogen, phosphorus, potassium, calcium, magnesium, manganese, and iron as influenced by the following factors: date of

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[2] In this discussion, "leaf or foliar analysis" refers to the quantitative analysis of the leaves, which involves considerations somewhat different from those involved in other types of plant-tissue tests.

[3] Schmidt, C. M., and Jameson, D. H. 1941 Bibliography of literature on analyses of leaf and other plant tissues, with special reference to content of mineral nutrients, 1935 through 1940. [Mimeographed] Amer. Potash Inst., Inc., Washington, D. C.

sampling; position of leaf on shoot; fruiting and nonfruiting terminals on the same trees; soil conditions; and fertilizer treatment.

METHODS

Each sample of leaves consisted of a composite of six or more normal leaves from the same position on the shoots from each of eight or more average bearing trees. Two leaves from each of three or more shoots were collected from each tree. The petioles were removed and discarded. Abnormally long or short shoots were avoided. The samples were dried at 70°C. as soon as possible after collection and then were ground in a Wiley mill using a 20-mesh screen. Preliminary analyses indicated that the contamination of the samples with iron by the mill used in this laboratory was too slight to be of importance in this investigation. Nitrogen was determined by the Kjeldahl-Gunning method. For the other determinations 10-gm. samples of the ground leaves were ashed in platinum dishes in a muffle furnace, with automatic temperature control, for about 7 hours at about 480°C. This length of time was found to be sufficient for satisfactory ashing of tung leaves. The ash was carefully moistened with a few cubic centimeters of water run down the side of the dish and then was transferred quantitatively to a 250-cc. beaker with the use of a wash bottle. Ten cubic centimeters of concentrated HCl was added while a watch glass was placed on the beaker to prevent loss from spattering. The solution was digested for about 2 hours on the steam bath, the small amount of silica and unburned carbon filtered off, and the filtrate made up to a volume of 100 cc. Suitable aliquots were taken for the various determinations.

Calcium and magnesium were determined in the same aliquot after removal of the R_2O_3 group plus manganese and phosphorus. Calcium was precipitated as the oxalate, which was titrated with standardized $KMnO_4$. Magnesium was determined by precipitating magnesium ammonium phosphate and titrating with standard acid. Phosphorus was determined colorimetrically by the molybdate blue method. Potassium determinations were made by the procedure of Hibbard and Stout (13) modified by the substitution of ceric sulfate for potassium permanganate as the oxidizing agent. Manganese and iron were determined colorimetrically as the permanganate and thiocyanate, the latter according to the method proposed by Winsor (29).

METHOD OF EXPRESSING RESULTS

Considerable discussion has arisen in the literature as to the best way to express the mineral content of plant tissue. Thomas (25) uses the "percentage of dry matter" basis and includes this concept in his definition of foliar diagnosis. Others (2) have pointed out the advantages in using the "per plant" or "per plant part" basis instead of the percentage basis. James (14) suggests that the different methods of expressing the mineral content of plants in studies of mineral nutrition must be considered in relation to the objectives. In his work both methods are used, the choice depending on the nature of the problem.

In normal seedling tung trees there is such great variation in the size and thick-

ness of similar types of leaves that differences in composition expressed on a per leaf basis are obscured. Furthermore the sampling procedure in this investigation did not presume a sample of leaves representative of the growth status of the tree. In cases where the sample accurately reflects the growth condition it would undoubtedly be advantageous to use the per leaf basis. In this study, however, the percentage basis is thought to be a precise criterion of the nutrient level of the tree.
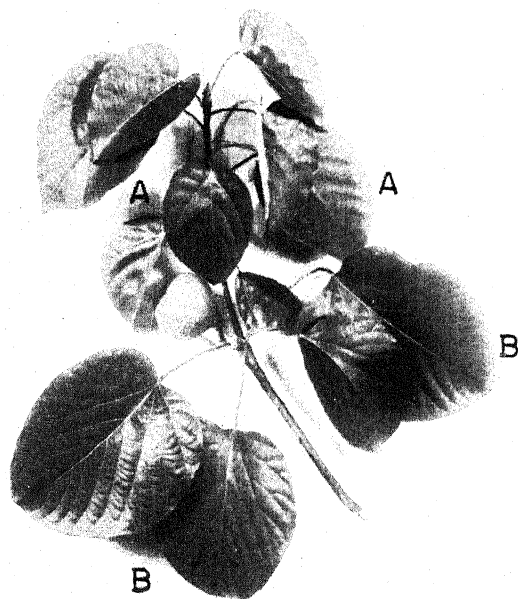

FIG. 1. BEARING TUNG SHOOT
A, midshoot leaves; B, basal leaves

ANALYSIS OF LEAVES COLLECTED IN THREE SEASONS TO DETERMINE INFLUENCE OF POSITION OF LEAF ON FRUITING AND ON NONFRUITING TERMINAL SHOOTS

Samples were collected in May, August, and October, 1941 from 8-year-old bearing tung trees in an orchard near Morriston, Florida. The soil type was principally Norfolk fine sand. The block from which the samples were collected is part of a fertilizer experiment that had been conducted by the U. S. Field Laboratory for Tung Investigations at Gainesville, Florida, for 3 years preceding this investigation. Composite samples were taken from three replicate plots of 15 trees each, all of which had received 300 pounds per acre of 4–0–3 mixed fertilizer. In most of the plots some trees were abnormally large or small or were defective, and these trees were avoided in sampling. At least eight trees bearing a light to moderate crop were sampled in each plot. The following four types of leaves were taken, two from each of three shoots from each tree: midshoot leaves

from fruiting terminals, midshoot leaves from nonfruiting terminals, basal leaves from fruiting terminals, and basal leaves from nonfruiting terminals (fig. 1). The same type leaves from each plot were composited. The analytical data were calculated on the basis of percentage of dry matter and were analyzed statistically by analysis of variance. Values for the average of the three replications are given in table 1.

Seasonal trends

There are highly significant seasonal trends in the concentration in the leaves of nitrogen, potassium, calcium, manganese, and ash, but no definite trend is indicated for magnesium, phosphorus, or iron. The percentage of calcium, manganese, and ash increases in the leaves from May to August and from August to October, whereas potassium and nitrogen decrease, though the decrease in the latter between May and August is not significant.

A decrease in the percentage of nitrogen and potassium in leaves as the season advances appears to be the result of a normal physiological process in most plants under ordinary conditions. This has been demonstrated for tree crops by a number of investigators (17, 18, 21, 23). Potassium-deficiency symptoms in tung leaves are most noticeable in the latter part of the growing season. As the mature tung fruit contains a relatively high percentage of potassium, almost 2 per cent of the dry weight,[4] it is to be expected that there would be a large migration in bearing trees of such a mobile element as potassium from the leaves into the fruit, although the withdrawal of potassium from the leaves undoubtedly also occurs in nonbearing trees.

An increase of calcium and manganese in leaves as the season advances has been reported by McHargue and Roy (18) for a large number of deciduous trees. Both of these elements are relatively nonmobile and tend to accumulate in leaves. Lilleland and Brown (17) found that calcium increased with age in the leaves of a number of fruit trees. The increase in the manganese content of leaves later in the season correlates with observations that manganese-deficiency symptoms in tung leaves tend to clear up in the latter part of the growing season.

Position of leaves

There is a highly significant difference between midshoot and basal leaves in the concentration of all constituents determined. The basal leaves, of course, are the first to appear in spring and are considerably older than the midshoot leaves at any one sampling date. It would be expected, then, that the content of the different elements in the midshoot leaves as compared to the basal leaves at any one sampling date would have the same relationship as the composition of leaves collected early in the season as compared to those collected late in the season. This is true of potassium, which is significantly lower in the basal leaves than in the midshoot leaves, and of calcium and manganese, which are significantly higher. This correlates with the fact that potassium-deficiency symptoms

[4] Sell, H. M., Best, A. H., Reuther, W., and Drosdoff, M. (In preparation.) Changes in the chemical composition of tung fruit during development.

TABLE 1

Mineral constituents of basal and midshoot tung leaves from fruiting and nonfruiting terminals collected on three sampling dates in 1941 from an 8-year-old orchard near Morriston, Florida*

CONSTITUENTS ON DRY-MATTER BASIS†

SAMPLING DATE	POSITION OF LEAVES	N		P		K		Ash		Ca		Mg		Mn		Fe		DRY WEIGHT PER LEAF	
		NF‡	F‡	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	p.p.m.	p.p.m.	p.p.m.	p.p.m.	gm.	gm.
May	Midshoot	1.88	1.88	0.17	0.17	1.18	1.20	5.37	5.77	0.86	0.96	0.30	0.33	22	22	41	51	0.64	0.42
	Basal	2.16	2.07	0.14	0.15	0.93	0.95	8.59	9.31	2.25	2.38	0.47	0.51	78	69	62	63	1.93	1.40
August	Midshoot	2.17	1.99	0.15	0.15	0.84	0.83	7.71	8.29	2.04	2.28	0.37	0.37	60	47	47	51	1.02	0.69
	Basal	1.92	1.66	0.13	0.14	0.66	0.70	10.92	11.42	3.10	3.30	0.55	0.54	109	73	62	61	1.89	1.64
October	Midshoot	1.95	1.73	0.16	0.16	0.70	0.72	8.44	9.34	2.36	2.73	0.36	0.34	77	94	49	53	1.00	0.72
	Basal	1.65	1.43	0.13	0.14	0.56	0.61	11.47	12.19	3.55	3.83	0.48	0.48	120	119	56	56	1.96	1.82

* The predominant soil type in this orchard is Norfolk fine sand. All trees in this experiment have received a 4-0-3 fertilizer in the past 3 years at the rate of 800 pounds per acre annually.

† Average of three replications.

‡ NF refers to leaves from nonfruiting terminals; and F, to leaves from fruiting terminals on the same tree.

of tung leaves generally develop first on the basal leaves (10) whereas manganese-deficiency symptoms first appear in the younger leaves.

For nitrogen there is a highly significant interaction between season and position of leaves. At the first sampling date in May the basal leaves were higher in nitrogen than the midshoot leaves, but as the season progressed the concentration of nitrogen in the older leaves became less than that in the younger leaves. This suggests that proteolysis occurs in the older leaves and the nitrogen is translocated to the growing points in the latter part of the growing season. Chibnall (7) discusses the question of proteolysis and translocation of nitrogen from the older to the younger leaves and cites evidence in support of this hypothesis. There was no significant change in the dry weight of the basal leaves during the season; hence, changes in percentage of nitrogen must have been largely due to translocation. With midshoot leaves, however, there was a significant increase from May to August in dry weight per leaf, which complicates the relationships due to the opposing effects of growth and absorption on the concentration of the nutrient in the leaf.

The magnitude of the differences in composition between the basal and midshoot leaves emphasizes the necessity of sampling similar type leaves in any diagnostic work using leaf analyses. With magnesium, phosphorus, and iron there is no significant seasonal trend, but there is a highly significant difference between the basal and midshoot leaves. The basal leaves are higher than the midshoot leaves in magnesium and iron and lower in phosphorus.

Fruiting versus nonfruiting terminals

In a comparison of the composition of leaves from fruiting and nonfruiting terminals on the same tree, only the percentages of nitrogen and calcium show consistently significant, though small, differences. The nitrogen is lower in the leaves from the fruiting terminals than in those from nonfruiting shoots, possibly because of a greater breakdown of the proteins and translocation of nitrogen to the fruit (7). With calcium the reverse is indicated, a higher percentage being found in the bearing terminals than in the nonbearing. The reason for this is not readily apparent. One possible explanation is that more proteolysis may occur in leaves of fruiting terminals and more oxalic or other organic acids may be formed to precipitate and immobilize the calcium (19).

In general, the differences in composition between leaves from bearing terminals and those from nonbearing terminals on the same trees are not large and for diagnostic purposes may be disregarded. As a general practice, however, in collecting samples for analyses the procedure now being followed is to take midshoot leaves from one or the other type of terminal, depending on the nature of the problem.

LEAF COMPOSITION ON DIFFERENT SAMPLING DATES DURING THE GROWING SEASON AS INFLUENCED BY SOIL CONDITIONS AND FERTILIZER TREATMENT

Samples of basal leaves from nonfruiting terminals were collected in July, August, and October from 7- to 9-year-old bearing trees in three orchards in widely scattered sections of the tung belt and on dissimilar soil types, as shown in table 2. The plots from which the samples were taken in all three orchards

TABLE 2

Mineral constituents of basal tung leaves from nonfruiting terminals collected on three sampling dates in 1941 from trees receiving high and low fertilizer applications in three different orchards 7 to 9 years old on three different soil types

CONSTITUENTS ON DRY-MATTER BASIS*

ORCHARD	SAMPLING DATE	N		P		K		Ash		Ca		Mg		Mn		Fe	
		L†	H‡	L	H	L	H	L	H	L	H	L	H	L	H	L	H
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Florida No. 1 Soil predominantly Norfolk fine sand	July	1.96	2.18	0.13	0.14	0.77	0.80	9.25	9.43	2.70	2.60	0.51	0.46	60	68	56	55
	August	1.63	1.76	0.11	0.12	0.67	0.76	9.95	10.46	2.92	3.20	0.49	0.41	58	70	40	57
	October	1.42	1.53	0.11	0.12	0.53	0.62	10.14	10.96	3.18	3.43	0.45	0.39	72	81	40	46
Florida No. 2 Soil predominantly Ruston fine sandy loam	July	2.30	2.38	0.14	0.14	0.89	0.89	10.90	10.78	3.04	2.99	0.57	0.58	638	956	80	88
	August	2.01	2.15	0.12	0.14	0.92	0.90	11.60	10.24	3.30	3.07	0.60	0.54	768	1147	75	87
	October	1.83	2.01	0.14	0.13	0.93	0.91	11.35	11.08	3.23	3.14	0.50	0.49	872	1314	76	72
Louisiana No. 1 Soil predominantly Pheba fine sandy loam	July	1.35	1.76	0.08	0.10	0.68	0.71	8.41	8.55	2.22	2.18	0.59	0.55	1625	1991	72	55
	August	1.08	1.27	0.08	0.09	0.49	0.57	8.80	8.78	2.47	2.39	0.62	0.56	2130	2351	75	71
	October	1.12	1.20	0.08	0.09	0.46	0.55	9.85	9.70	2.73	2.66	0.64	0.55	2633	3110	76	73

* Average of three replications.

† L refers to trees in plots that have received in the past 3 years the respective fertilizer treatments indicated: Florida orchard No. 1, 300 pounds per acre of 4–0–3 fertilizer; Florida No. 2, 400 pounds of 4–4–3; Louisiana No. 1, 300 pounds per acre of 4–4–3 in 1939 and 1940, and none in 1941.

‡ H refers to plots that have received 300 pounds per acre of 12–12–9 fertilizer in the past three years (Florida No. 1 and Louisiana No. 1), or 400 pounds per acre of 12–12–9 (Florida No. 2).

are parts of fertilizer experiments that have been conducted during the 3 years preceding the year of sampling at the Bogalusa, La., Cairo, Ga., and Gainesville, Fla. U. S. Field Laboratories for Tung Investigations. The trees receiving high and low fertilizer applications in each orchard were sampled separately. The low fertilizer treatment was 300 pounds per acre of 4–0–3 at Florida orchard No. 1, 400 pounds of 4–4–3 at Florida No. 2, and 300 pounds of 4–4–3 in 1939 and 1940 and none in 1941 at Louisiana orchard No. 1. The high fertilizer treatment consisted of 300 pounds per acre of 12–12–9 at Florida orchard No. 1 and Louisiana No. 1 and 400 pounds per acre of 12–12–9 at Florida No. 2. The orchard management practices in the three orchards were reasonably comparable. The sampling procedure was the same as that given in the preceding section. The data for the analyses were calculated on a percentage of dry matter basis, and the values for the averages of three replications are given in table 2. The data were analyzed statistically by analysis of variance.

Orchard location

The difference in leaf composition due to orchard location are outstanding. Neither the fertilizer treatments nor the date of sampling caused such wide differences in the composition of the leaves as did the soil on which the trees were growing. Though there are some differences between the three orchards in management and climate and possibly other factors, the most outstanding differences are due to the differences in the soils. The three predominant soil types of these orchards, Norfolk fine sand, Ruston fine sandy loam, and Pheba fine sandy loam, are distinctly different in most of the important soil properties. Norfolk fine sand, in Florida orchard No. 1, is a very light textured soil containing not more than 5 per cent clay, and is low in organic matter, the surface soil containing about 1 per cent. This soil is subject to excessive leaching. Pheba fine sandy loam, in Louisiana orchard No. 1, is a relatively heavy textured soil, especially in the subsoil, which usually contains over 30 per cent clay. The organic matter content of the surface soil is high, generally over 3 per cent. This soil has a compact subsoil layer which restricts drainage considerably during periods of high precipitation. This causes insufficient aeration for the tree roots and seriously impedes absorption of nitrogen and the mineral elements. Ruston fine sandy loam soil, in Florida orchard No. 2, is considered one of the best soils for tung trees as well as for most crops in the South. It has a loamy sand to sandy loam surface containing 2 per cent or more organic matter. The brown sandy clay subsoil is well drained and aerated.

The trees in Florida orchard No. 2 on the Ruston soil have the highest, and those in Louisiana orchard No. 1 on the Pheba soil have the lowest concentration of nitrogen, phosphorus, potassium, and calcium in the leaves, regardless of fertilizer treatment. These differences are reflected in the general vigor and growth of the trees in the two orchards. Florida orchard No. 2 is one of the best producers in the tung belt, whereas Louisiana orchard No. 1 is relatively poor. Even those trees in Louisiana orchard No. 1 receiving a 12–12–9 fertilizer at the rate of 300 pounds per acre show an abnormally low content of

nitrogen, phosphorus, and potassium in the leaves. Undoubtedly the lack of response by the trees in Louisiana orchard No. 1 to the heavier fertilizer application can be explained by the lack of aeration in the soil due to the poor, impeded drainage conditions.

The difference in manganese content of the leaves from the three orchards is striking. Florida orchard No. 1 shows symptoms of manganese deficiency and, therefore, the manganese content of the leaves may be considered inadequate. The high manganese content of the leaves from the other orchards would appear to indicate luxury consumption. Although the leaves from Louisiana orchard No. 1 may contain more than 3000 p.p.m. of manganese, there was no indication of manganese toxicity. The soil in this orchard is very acid and apparently contains a relatively high amount of soluble manganese. The range in leaf content of manganese from 58 p.p.m. in Florida orchard No. 1 to 3110 p.p.m. in Louisiana orchard No. 1 greatly exceeds the range for any other element.

The difference among orchards in the iron content of the leaves is highly significant statistically but the range is not great. Although Florida orchard No. 2 and Louisiana orchard No. 1 have a relatively high content of total iron oxide in the highly acid subsoils, it is surprising to find that the iron content of the leaves from these orchards is not greatly different from that of leaves from Florida orchard No. 1, which has a slightly acid sandy soil with a very low iron content.

Seasonal trends

In general, the same seasonal trends are indicated in the basal leaves from all three orchards as was noted in the preceding section in the discussion of table 1. Calcium, manganese, and ash show a highly significant increase in percentage as the season advances, and nitrogen and potassium show a decrease. Florida orchard No. 2 is exceptional, however, in that the potassium content of the leaves is maintained throughout the season. A possible explanation is that in the past this orchard has been heavily fertilized with potash, the level of which in the soil may be sufficiently high to maintain the level in the leaves throughout the growing season.

Fertilizer treatment

The increase in the nitrogen content of the leaves due to the high fertilizer treatment is highly significant, and though the magnitude of the increase is not great it is consistent in all three orchards. Although the greater phosphate content of the leaves with the heavier fertilizer treatment shows high statistical significance, the amount of the increase is hardly deemed important. The increase found in the amount of potassium in the leaves due to the higher fertilizer treatment did not quite reach significance at the .05 level even though the potassium content of the leaves in both Florida orchard No. 1 and Louisiana orchard No. 1 is low. This is understandable at Louisiana orchard No. 1 where insufficient soil aeration is undoubtedly an important factor restricting absorption. The situation in the Florida orchard No. 1 is less clear. In general, the trees in this

orchard are not making good growth, and copper- and manganese-deficiency symptoms are apparent.

SUMMARY

Analyses were made of leaves collected on three dates during the growing season from bearing tung orchards in different parts of the tung belt to determine the effect on leaf composition of: (a) the position of the leaf on the terminal shoot, (b) the presence of fruit on the terminal, (c) soil conditions and fertilizer treatment. Nitrogen, phosphorus, potassium, calcium, magnesium, manganese, iron, and ash were determined.

It was found that the percentages of calcium and of manganese increase as the season advances whereas potassium and nitrogen decrease. No definite seasonal trend is indicated for magnesium, phosphorus, or iron.

The percentage of potassium is significantly lower in the basal leaves than in the midshoot leaves, and calcium and manganese are significantly higher. At the first sampling date in May the basal leaves are higher in nitrogen than the midshoot leaves, but at the later sampling dates the midshoot leaves are higher.

Nitrogen is lower in leaves from fruiting terminals than in those from similar but nonfruiting shoots. For calcium the reverse is indicated.

Differences in leaf composition due to the nature of the soils on which the orchards are situated are greater than those caused by any other factor involved in these studies.

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PURIFICATION OF WATER BY USE OF SYNTHETIC ION-EXCHANGE RESINS: USING pH AS A CONTROL

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Synthetic resins have been employed as adsorbents for considerable time, and recently their use in water purification has received attention. Myers, *et al.* (4, 5, 6) demonstrated the ability of synthetic ion-exchange resins to adsorb cations and anions from aqueous solution. Their work involved the efficiency of the adsorption of ions and the necessary regeneration of the resins and was conducted on a resin known as "Amberlite." Myers and Eastes (5) suggested the use of pH determinations in detection of the break-through or saturation point. Myers, Eastes, and Urquhart (6) reported that the synthetic ion-exchange resins exhibited typical Freundlich adsorption isotherms when in contact with acid and salt solution and that the break-through capacity may be calculated from these isotherms. Harrisson, Myers, and Herr (2) found that the use of synthetic resins in water purification would produce water equal in quality to that required by the U. S. P. standards for distilled water. They also gave an illustration of a simple laboratory set-up for this purpose. Liebig, Vanslow, and Chapman (3) found that water passed through synthetic resins could be further purified by repassage. They were able to grow citrus seedlings successfully in nutrient solutions using water produced in this manner. The Resinous Products and Chemical Co. (7) presented detailed data on the adsorption capacity and stability of the synthetic resins produced by them and on the cycles that may be used in their operation. Their work was on "Amberlites," of which IR_1 and IR_{100} were cation exchangers and IR_4 was an anion exchanger. A diagram of a simple laboratory set-up was also presented.

The water output from laboratory stills is often too low for nutrient solution work, and the copper content of distilled water from copper stills is too high for general laboratory procedures. For these reasons the authors became interested in the use of ion-exchange resins for the purification of water.

MATERIALS AND METHODS

Two sets of "Amberlite" towers were constructed in accordance with the design of laboratory apparatus presented by the Resinous Products and Chemical Co.

(7), with slight modifications. One set contained IR_1 as a cation exchanger, and the other contained IR_{100}. Both these cation exchangers were used in the hydrogen form. Both sets contained IR_4 as an anion exchanger. Tap water was passed through these towers.

Quantitative spectrographic analyses were made for copper, lead, and zinc on the samples of water. One liter of each sample was concentrated to 30 ml., the pH made slightly alkaline, and the metallic content extracted with dithizone. A measured amount of palladium dithionate was added as an internal standard. The quantities present were calculated in terms of intensity ratios on calibrated plates.

Comparative spectrographic analyses were made for iron, manganese, magnesium, sodium, and calcium. A liter of each sample was concentrated to 30 ml., and 0.5 ml. of the concentrate was dried on electrodes and spectrographed in duplicate. A visual comparison was made with standards similarly prepared, and the results were converted to parts per million of these elements.

Conductometric methods were used in determining total concentration of soluble salts. The conductivity bridge used conformed to specifications of the U. S. Bureau of Soils, and the temperature corrections and conversions of resistance values to parts per million of soluble salts were made from tables for this bridge (1). In order to obtain a readable resistance value for several of these samples, it was necessary to add a known quantity of impurity. A homogeneous sample of tap water was used for this purpose.

EXPERIMENTAL DATA AND DISCUSSION

The set of towers containing IR_1 was constructed first. These towers contained 628 cc. of IR_1 and 581 cc. of IR_4. This amount of "Amberlite" filled a 6.5-cm. tower to heights of 40 and 37 cm. respectively as a backwashed and drained volume. A test of pH was considered a good indication of the breakthrough point reached upon saturation of the IR_1. Tap water was flowed through these towers at rates of 250, 500, 750, and 1,000 cc. per minute in order to determine the efficiency of operation. The water was tested at intervals of 7.5 liters as it came from the IR_1 tower, and the pH was found to remain at 3.0; after passing through both the IR_1 and the IR_4 tower, the water had a pH of 4.5; and when boiled to remove carbon dioxide, the final water had a pH of 6.8 to 7.0. These pH values were constant until the IR_1 became saturated, at which point the pH rose rapidly. This occurred after 82.5 liters had passed through. A pH of 5.0 in the final water was considered to be the break-through point.

Regeneration of the resins at the end of each cycle was conducted on the volumetric basis as suggested by the Resinous Products and Chemical Co. (7) using 4 per cent HCl for IR_1 and 4 per cent Na_2CO_3 for IR_4. The pH of the effluent after the required amount of regeneration solution was passed through the resins was 0.5 to 1.0 for IR_1 and 10.0 to 10.5 for IR_4. After the regenerates were passed through, the resins were washed with water previously purified by use of these resins until the effluent showed a pH of 2.5 for IR_1 and 6.5 for IR_4.

The break-through point for all rates was very sharply defined. After several

cycles which gave the same pH and volume relations, these towers were operated on a volume basis, pH determinations being used as a frequent check on the regeneration and purification processes. An automatic overflow was installed to prevent water from entering the containers after the break-through point was reached.

In table 1 are presented the pH of de-ionized water at time of sampling, rate of flow through towers, and the concentration of soluble salts in the sample. Tap, distilled, and redistilled water are used for comparison.

As is readily seen in table 1, when the pH was below 5.0 the concentration of the soluble salts in the de-ionized water was much less than that of redistilled water. The only readings for soluble salts obtained on "Amberlite"-treated water, while the pH was 3.0 and 4.5 for IR_1 and IR_4 respectively, was obtained

TABLE 1

Concentration of soluble salts in de-ionized water, as influenced by rate of flow, as compared with tap, distilled, and redistilled water

WATER SAMPLE	RATE OF FLOW	pH		SOLUBLE SALTS
		IR_1	IR_4	
	cc./min.			*p.p.m.*
Tap water*..............				262.5
Distilled................				2.9
Redistilled.............				1.0
Amberlite 1.............	1,000	3.0	4.5	0.1
Amberlite 2.............	1,000	3.3	5.4	9.8
Amberlite 3.............	750	3.0	4.5	0.1
Amberlite 4.............	750	3.3	5.3	59.8
Amberlite 5.............	500	3.0	4.5	0.0
Amberlite 6.............	500	3.7	5.3	87.0
Amberlite 7.............	250	3.0	4.5	0.0
Amberlite 8.............	250	5.3	5.9	120.6

* pH of tap water ranged from 7.0 to 7.5.

with a flow of 750 and 1000 cc./min. At these two rates of flow considerable difficulty was encountered in preventing the stoppers from blowing out of the towers. A comparison of the pH values for IR_1 and the soluble salts for the even-numbered samples indicates a direct relationship between the pH and amount of soluble salts present. The slower rates of flow had a much sharper break-through point than the faster rates of flow.

For spectrographic analysis another set of samples was collected. These consisted of two samples of water treated with IR_{100} and IR_4, four samples treated with IR_1 and IR_4 with the pH at 3.0 and 4.5 respectively, tap water, distilled water, and redistilled water. Table 2 presents the analysis of these samples for zinc, copper, lead, iron, calcium, manganese, magnesium, and sodium.

The data in table 2 show that all cations were reduced when the water was passed through "Amberlite." Since all cations were reduced below the level of that of distilled water except for the magnesium, sodium, and calcium content

of water treated with IR_{100}, indications are that the "Amberlite" in these towers may not have been completely regenerated and washed. Also, the two samples treated with IR_{100} were collected from the first cycle of these towers. The abnormally high magnesium content of these samples indicates that the samples may have been contaminated. Such concentrations of magnesium should not occur in subsequent cycles with proper manipulation. The data indicate that water passed through "Amberlite" is comparable to redistilled water in regard to cation content and is equal to redistilled water in regard to copper content.

The four samples of water treated with IR_1 in table 2 were obtained without regeneration of IR_4. Apparently the use of IR_4 until it becomes saturated has no harmful effects on the quality of the water. The pH of the final water increased approximately 0.1 for each cycle after regeneration of IR_4.

TABLE 2

Spectrographic analysis* of cations in de-ionized water as compared with tap, distilled, and redistilled water

SAMPLE	Zn	Cu	Pb	Fe	Ca	Mn	Mg	Na
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Tap....................	0.02	0.0070	0.002	0.6	6.0	0.02	10.0++	5.0
Distilled†..............	0.02	0.1400	0.010	0.12	0.3	0.01−	10.0+	0.2
Redistilled‡............	0.00	0.0014	0.000	0.01−	0.01−	0.01−	0.01−	0.01−
Amberlite 1 (IR_{100}).....	0.00	0.0003	0.000	0.01−	0.3	0.01−	10.0+	0.2
Amberlite 2 (IR_{100}).....	0.00	0.0014	0.005	0.03	1.2	0.01−	10.0+	1.0
Amberlite 9 (IR_1).......	0.01	0.0035	0.000	0.03	0.01−	0.01−	0.01−	0.01−
Amberlite 10 (IR_1).......	0.01	0.0008	0.000	0.01−	0.01−	0.01−	0.01−	0.01−
Amberlite 11 (IR_1).......	0.01	0.0016	0.002	0.03	0.01−	0.01−	0.01−	0.01−
Amberlite 12 (IR_1).......	0.00	0.0017	0.000	0.03	0.01−	0.01−	0.01−	0.01−

    * ++ indicates amount present much greater than value given; +, amount present greater than value given; −, amount present less than value given.
    † Obtained from a copper still.
    ‡ Obtained from a glass still using distilled water.

The copper content of the water samples used in table 1 was tested by use of sporangia of *Phytophthora infestans* Thax. (Guss.), the germination of which is greatly reduced by the presence of small quantities of the element. The sporangia showed 1 per cent germination in tap water, 40 per cent in distilled water, and 95 per cent in redistilled water; in samples of "Amberlite"-treated water collected when the pH was 3.0 and 4.5 (odd-numbered samples in table 1) they showed an average of 65 per cent germination, and in those collected when the pH was above 5 (even-numbered samples in table 1), an average of 69 per cent germination. The germination of the sporangia indicated that the copper content of treated water was lower than that of distilled water but not so low as that of redistilled water. It also indicated that there is a continued adsorption of copper after the break-through point for the cation exchanger has been reached. The greater germination of the sporangia in distilled water as com-

pared to tap water may be due to the presence of soluble salts other than copper in tap water. The effects of other soluble salts upon germination is unknown. Also the presence of small quantities of dissolved resins may have resulted in a germination percentage lower for de-ionized water than for redistilled water.

SUMMARY

The use of synthetic resins such as "Amberlite" in the purification of water appears to be very promising. The water treated with these ion-exchangers is comparable and in many respects superior to distilled water, whereas in several respects the de-ionized water is very comparable to redistilled water.

Conductivity determinations indicate that the break-through point for the towers may be determined by the use of an ordinary laboratory model pH meter. Such a meter may also be used to regulate the regeneration and washing process after each cycle.

Germination of sporangia of *Phytophthora infestans* Thax. (Guss.) indicated that there is a continued adsorption of copper after the cation exchanger is saturated and also that the copper content of the water is lower than that of distilled water but not so low as that of redistilled water.

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SOME FACTORS AFFECTING CHLOROSIS ON HIGH-LIME SOILS: I. FERROUS AND FERRIC IRON

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Chlorosis on high-lime soils is generally due to a deficiency of either iron or manganese. In Utah this type of chlorosis has been attributed to iron deficiency because chlorotic fruit trees have responded to injections and sprays of iron salts but have shown no noticeable improvement from treatment with manganese (33). Iron deficiency in plants has been widely associated with a high lime content of soil, or with poor drainage and poor aeration. The present studies are confined to chlorosis on high-lime soils without water tables or impeded drainage. The purpose of the paper is to consider some of the factors influencing iron absorption and utilization by plants.

The extensive literature concerning iron chlorosis on high-lime soils shows no consistent agreement on whether chlorosis results from iron insolubility in soils, thus preventing adequate absorption by the plant, or whether it results from unbalanced physiological conditions within the plant preventing iron transport and utilization. Both points of view will be considered.

REVIEW OF LITERATURE

Factors affecting iron solubility in soils

The role of lime in inducing chlorosis is complicated by the fact that there is no consistent difference in the lime content of soils where plants are green and where they are chlorotic, even though such differences may exist within limited areas (14, 30). Some investigators have held that interactions between lime and other soil constituents may account for iron insolubility. Buehrer and Williams (3) have shown that the hydrolysis of calcium carbonate in high-lime soils results in high pH values. This theory has been applied to iron solubility (12, 30) by showing that interactions between lime and moisture increased soil pH and that this is related to iron solubility in soils. The latter investigations showed a good correlation between pH of dilute soil suspensions and the incidence of chlorosis. This is in agreement with several studies showing that chlorosis and the pH of high-lime soils are related to soil texture, profile characteristics, and moisture content (4, 11, 13).

Most studies of chlorosis have not attached much significance to the form in which iron is absorbed and utilized by plants, but several recent papers (17, 28) have stressed the importance of ferrous iron in plant nutrition. Relationships between ferrous and ferric iron in high-lime soils have not been adequately investigated. Brewer and Carr (2) reported that ferrous iron is not readily precipitated as the hydroxide and is available to plants in low-lime soils with pH values as high as 8.0. Willis (34), also working with low-lime soils, showed that

an increase in reducing conditions increases the solubility of iron.   He believes that much of the beneficial influence of organic matter on plant growth is due to the promotion of a more favorable Eh in the soil.   Kliman (17) asserts that iron can be absorbed by plants in only the ferrous form and, like Willis, gives evidence in support of soil organic matter as a soil-reducing system.   Chapman (5) found that in high-lime soils organic matter brings iron into solution but that calcium carbonate is continually removing it from the solution.   The concept that organic matter increases iron solubility is opposed, however, by observations in Utah that treatment of high-lime soils with farm manure frequently increases the amount and severity of chlorosis.

Plant characteristics associated with chlorosis

A number of studies have reported the comparative analysis of chlorotic and green plants.   Most investigators (5, 8, 10, 20, 22) have found slightly more iron in green leaves, but Allyn (1) and Wallace and Mann (31) observed no distinct difference in composition.   All results were reported on dry weight, green weight, or ash basis.   Oserkowsky (22) found no correlation between total iron and chlorophyll in leaves.   He concluded that total iron in plants is of little significance but that active iron in plants is directly related to chlorophyll formation, where active iron is included in the iron soluble in N HCl.   Undoubtedly much of the iron in plants is present in inactive forms.

In some investigations iron deficiency in leaves has been related to low-potassium nutrition.   Cullinan et al. (6) observed such a result with peach trees grown in nutrient solutions.   Allyn (1) found that potash and manure treatments on limed soils decreased deposition of iron at the nodes of corn.   Observations with corn have led to the theory that potassium facilitates iron movement in plants (1).   On the basis of such studies and a few leaf analyses, some of the earlier writers in plant physiology attributed iron chlorosis to the prevention, by potassium deficiency, of iron transport within plants (8).   Most studies of chlorosis on high-lime soils indicate, however, a greater concentration of potassium and a lower concentration of calcium in chlorotic leaves than in green leaves (9, 10, 19, 31, 32).   Such results have been interpreted as indicating an antagonism between iron and potassium in chlorophyll formation (24).   But Oserkowsky (21) found no difference between potassium and iron concentrations in tracheal sap of green and chlorotic branches from the same orchard.

In several studies an inverse relationship has been found between the pH and the iron content of sap expressed from leaves and branches (16, 21, 23).   Rogers and Shive (23) suggest that the numerous exceptions to this generalization might be explained by the presence of larger amounts of organic iron solvents in the sap of some plants.

The close relationship between iron chlorosis and the manganese nutrition of plants is widely recognized.   This relationship is well illustrated by the recent work of Sommers and Shive (29) in which plants with a constant source of iron were chlorotic, green, or chlorotic as the manganese level of the nutrient solution was varied from low to medium to high, respectively.   High ratios of manganese to iron apparently induce chlorosis by oxidizing iron to the more in-

soluble ferric forms.   Plants growing on high-lime soils usually have moderate to low content of manganese (5), but the ratios of manganese to iron in available forms in high-lime soils or in active forms within plants are not known.

It has been suggested (5, 25, 28, 29) that frequently the oxidation-reduction systems of chlorotic plants may be out of balance, resulting in the oxidation of ferrous to ferric iron and a subsequent iron precipitation.   This theory is in harmony with the conclusion of Kliman (17) that only reduced iron is active in plants.

Recent suggestions of the importance of ferrous iron in soils and in plant nutrition indicate new approaches in the study of iron chlorosis.   Studies are in progress in this laboratory on relationships between ferrous and ferric iron and chlorosis, oxidation-reduction systems and potentials of soils and plants in relation to chlorosis, and relationships between organic matter and acidulated materials and iron availability in high-lime soils.   This report covers some of the data obtained in preliminary experiments related to the first phase of the projected investigations.

MATERIALS AND METHODS

Chlorosis is widespread on high-lime soils in fruit-producing areas of Utah. For preliminary studies paired samples of soils and leaves were obtained from closely adjacent areas where plants were green and where they were chlorotic. Soil samples were taken from the sides of freshly dug holes.   For leaf samples 100 leaves were obtained from the outer ends of current terminal twig growths of one tree, or of two or four adjacent trees, depending on the uniformity of the chlorotic condition.   Plants studied included peach, pear, prune, apple, grape, and honey locust.   All leaves were washed in distilled water.   Those for "sap" studies were dried of excess water between blotting papers and frozen, thawed rapidly, and put in a hydraulic press at 10,000 pounds' pressure.   The sap was further clarified by passage through fine filter paper, or for some purposes through Pasteur-Chamberland filters.   About 10 per cent of the leaves were selected at random from each sample.   Each leaf selected was traced on paper of uniform weight.   The tracings were cut out and the average weight per unit leaf area for each sample was calculated from the weights of the leaves and the weights of the tracings.

Iron was determined in the ferrous form by the method of Dyer and McFarlane (7) using a′a′dipyridyl.   Ferric iron was taken as that reduced by sodium hydrogen sulfite, except in a few studies where iron was determined directly in ferric form by the thiocyanate method.   Color comparisons were made in an Aminco Type F electrophotometer.   A Beckman glass electrode assembly was employed for pH determinations.   Other analyses were made in accordance with usual procedures, or according to later specifications.

RESULTS

Soil studies

In an earlier study it was found (30) that more iron is extracted by solutions of oxalic acid from soils producing green plants than from those producing chlo-

rotic plants. Later analyses of such extracts showed that the difference in extractable iron was due chiefly to greater amounts of ferrous iron in the extracts of soils producing green plants, but there was no appreciable difference in the ferric iron content of the soil extracts. This appeared to indicate a significant difference in ferrous iron content of the soils studied; but further tests indicated that oxalic acid will reduce solutions of ferric chloride to the ferrous state when organic materials are present, and that well-drained calcareous soils contain no determinable quantities of water-soluble or replaceable iron in ferrous form. The

TABLE 1

Iron and manganese soluble in 1–5 extracts of soil with normal ammonium acetate plus 0.2 per cent hydroquinone solutions at pH 5 and pH 7

ORCHARD	COUNTY	MANGANESE (pH 5)		MANGANESE (pH 7)		IRON (pH 5)	
		Chlorotic	Green	Chlorotic	Green	Chlorotic	Green
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1 Pear	Utah	121	140	43	75	0.3	12.0
2 Pear	Utah	117	152	38	60	3.2	7.5
3 Pear	Utah	151	163	62	60	10.0	10.0
4 Peach	Utah	120	140	43	50	2.5	8.7
5 Peach	Utah	189	134	84	67	1.3	6.8
6 Peach	Utah	111	117	34	117	1.1	2.9
7 Peach	Utah	127	151	23	31	2.6	6.0
8 Peach	Utah	127	132	52	91	1.7	4.0
9 Peach	Utah	83	142	29	72	1.2	2.5
10 Peach	Utah	133	127	42	36	0.9	4.6
11 Peach	Utah	129	147	55	45	1.0	1.5
12 Grape	Cache	86	115	32	59	6.8	16.9
13 Peach	Utah	113	159	45	67	6.4	4.8
14 Pear	Utah	110	128	44	47	4.0	4.6
Mean		122.6	139.1	44.7	62.6	3.07	6.63
Mean difference		16.5*		17.9*		3.56**	
Standard error of mean difference		5.67		6.58		1.01	

* Mean difference significant beyond the 5 per cent level.
** Mean difference significant beyond the 1 per cent level.

results did indicate, however, that iron in soils where plants are green may be more readily reduced than iron in soils of chlorotic areas.

Sherman *et al.* (26, 27) have related the availability of manganese in soils to that which is reduced by a normal solution of ammonium acetate (pH 7.0) containing 0.2 per cent hydroquinone. This method of analysis was applied to fresh samples of several surface soils. Some of the data obtained are shown in table 1. Since little iron was obtained with a pH 7.0 buffer, analyses were repeated with the ammonium acetate adjusted to pH 5.0. The extraction at pH 7 brought less manganese into solution but did not differentiate better than the extraction at

pH 5 between soils producing green plants and those producing chlorotic plants. The quantities of iron removed at pH 7 were usually too small for accurate determination.

The data of table 1 indicate that both iron and manganese were reduced significantly more by the hydroquinone solution in soils producing green plants than in soils producing chlorotic plants. No determinable quantities of either element were removed from the soils by the ammonium acetate solution without hydroquinone. The quantities of calcium and potassium extracted from soils by the ammonium acetate solution at pH 5 were also determined. The calcium contents of the extracts were consistently higher in the soil samples from chlorotic areas than in paired samples from green areas, but the quantities of potassium extracted showed no relation to chlorosis.

TABLE 2

Iron solubility as influenced by iron additions to soil

Normal ammonium acetate, pH 5, plus 0.2 per cent hydroquinone extracting solution

SOIL NUMBER	SOIL DEPTH	PLANT APPEARANCE	CaCO₃	pH SOIL PASTE	IRON EXTRACTED FROM SOILS		
					No Fe added	Fe⁺⁺ added*	Fe⁺⁺⁺ added*
	inches		*per cent*		*p.p.m.*	*p.p.m.*	*p.p.m.*
3	0–12	Chlorotic	21.5	8.04	7.8	10.6	9.1
	12–24	Chlorotic	46.4	8.09	5.0	16.1	10.0
3A	0–12	Green	6.2	7.62	8.8	14.2	12.1
	12–24	Green	37.3	7.82	7.8	16.9	13.5
5	0–12	Green	0.72	7.81	13.8	26.0	24.2
	12–24	Green	0.60	7.70	16.4	24.2	26.8
6	0–12	Green	0.00	6.80	13.8	25.8	21.5

* 200 mgm. of ferrous or ferric iron added as the chloride salt.

The influence of calcareous soils on soluble iron is illustrated in table 2. Iron in the form of dry chloride salts was mixed with fresh soil samples at the rate of 200 γ of iron per gram of soil. The soil was brought to about the moisture equivalent and kept at about 25° C. for 12 hours before extraction. Soils 3 and 3A are from adjacent areas differing in chlorosis. Soils 5 and 6 are from areas in which no chlorosis has been observed. The results indicate a rapid reversion of iron to forms not readily reducible by hydroquinone. The reversion was slightly less in the low-lime soils which do not induce chlorosis. Somewhat less iron was reduced by the hydroquinone in soils from chlorotic areas than in soils from adjacent areas of green plants.

Plant studies

No consistent difference has been found by various investigators between the total iron content of green and chlorotic leaves. Usually results have been ex-

pressed on a dry or a green weight basis. These methods fail to allow for the stunted growth of chlorotic plants. It appears to the authors that expression of leaf composition on an area basis makes better allowance for growth differences. Expression on an area basis does, however, introduce another error in that green leaves are frequently thicker than chlorotic leaves. In the present study expression of leaf composition on the basis of leaf area resulted in a greater difference between the composition of chlorotic and green leaves with respect to iron, manganese, and calcium but did not change the potassium relations appreciably. This is shown in table 3. On an area basis, the green leaves contained significantly more iron than the chlorotic leaves; but on a dry weight basis, the differences were not significant. For manganese and calcium the mean differ-

TABLE 3

Composition of chlorotic and green leaves

PLANT	PLANT APPEARANCE	NUMBER OF SAMPLES	IRON		MANGANESE		CALCIUM		POTASSIUM	
			p.p.m.	$\gamma/cm.^2$	p.p.m.	$\gamma/cm.^2$	per cent	$\gamma/cm.^2$	per cent	$\gamma/cm.^2$
Elberta peach....	Chlorotic	4	114	0.68	2.0	.012	1.22	71.5	0.599	37.3
Elberta peach....	Green	4	136	0.97	8.0	.061	1.57	109.5	0.232	17.3
Bartlett pear.....	Chlorotic	3	95	0.68	14.6	.102	1.57	116.7	0.194	13.5
Bartlett pear.....	Green	3	125	1.16	24.2	.211	2.11	163.0	0.103	9.5
Jonathan apple...	Chlorotic	1	158	0.65	16.7	.067	1.52	62.0		
Jonathan apple...	Green	1	169	0.86	30.9	.160	1.45	75.0		
Concord grape....	Chlorotic	1	138	0.73	15.6	.084	1.45	76.0		
Concord grape....	Green	1	104	0.79	53.5	.368	2.25	155.0		
Mean..........	Chlorotic		115.0	0.68	9.34	.056	1.40	86.1	0.438	27.9
	Green		132.4	1.00	21.2	.155	1.81	128.6	0.181	14.1
Mean difference.....................			17.4	0.32*	11.86*	.099**	0.41*	42.5**	.257	13.8
Standard error mean difference.......			10.84	0.102	3.68	.025	0.13	8.68	.100	6.07

* Significant beyond the 5 per cent level.
** Significant beyond the 1 per cent level.

ences for the dry weight basis were significant, but the differences were highly significant for expression on the area basis. The difference with respect to potassium would probably have been significant if as many samples had been analyzed as were tested for iron and manganese. Though the data presented on leaf composition indicate a significantly greater content of iron and manganese per unit of area in green leaves than in chlorotic leaves, it is believed the form and activity of iron are more important than the total.

Table 4 gives the average composition of current-year twig growth of peach and pear trees. Results among individual samples were variable, and consequently the differences in composition between twigs from chlorotic and from green trees are not significant. A distinct difference in the iron content of fruit is indicated, however, in table 5.

A number of analyses were made on the nitrogen content of leaves. In agreement with findings of several other investigators, the data showed that chlorotic leaves contained appreciably more ammonia nitrogen than green leaves, but there was no consistent difference between leaves in the content of nitrate nitrogen.

Solubility in various extracting solutions has been employed as a criterion of the mobility of iron in plants. Oserkowsky (22) found a positive correlation between percentage of chlorophyll in leaves and iron soluble in N HCl, but not between chlorophyll and total iron in leaves. Some results obtained in the present study with such HCl extractions are shown in table 6. These data, in agreement with the results of Oserkowsky, indicate a much greater content of HCl-

TABLE 4

Composition of terminal branches from chlorotic and green trees

Average values

VARIETY	LEAF APPEARANCE	IRON	MANGANESE	CALCIUM	POTASSIUM	SODIUM
		p.p.m.	p.p.m.	per cent	per cent	per cent
Elberta peach..........	Chlorotic	96	7.0	1.06	.035	.035
Elberta peach.........	Green	71	6.3	0.90	.027	.045
Bartlett pear...........	Chlorotic	81	8.0	0.78	.037	.045
Bartlett pear..........	Green	108	8.5	0.70	.041	.043

TABLE 5

Average iron content of fruit from chlorotic and green trees

Near maturity

VARIETY	PLANT APPEARANCE	NUMBER OF FRUITS ANALYZED	IRON PER KGM. GREEN WT.	IRON PER FRUIT
			mgm.	mgm.
Peach........................	Chlorotic	23	24.1	0.823
Peach........................	Green	25	36.5	2.062
Pear.........................	Chlorotic	13	14.8	1.683
Pear.........................	Green	11	21.8	2.225

soluble iron in green leaves than in chlorotic. A second extraction of the leaves in the same manner gave only traces of iron, indicating that the first extraction removed readily soluble iron from the plant tissues.

In later work the significance of ferrous iron in plant tissues became of interest, and similar extractions were repeated with various reagents, analyses being made on the extracts for ferrous and ferric iron. Representative results are shown in table 7. These data were obtained by extraction of Concord grape leaves by grinding them in a mortar under an oil film with silica sand and a 1:10 (green weight) dilution of the leaves with the solutions. Greater quantities of ferrous iron were extracted by the acid solutions from green than from chlorotic leaves. It was found, however, that like oxalic acid, these reagents are able to reduce ferric iron in the presence of certain organic materials. The quantities of iron

reduced in 1 per cent solutions of glucose and of starch are shown in table 8. Aluminum chloride and formic acid reduced the most iron in the presence of glucose, and formic acid brought about the greatest reduction in the presence of

TABLE 6

Iron extracted from chlorotic and green leaves by 1N HCl

Average values

| PLANT | PLANT APPEARANCE | NUMBER OF LOCATIONS | IRON EXTRACTED | | |
			Per gm. green wt.	Per gm. dry wt.	Per cm.^{2}
			γ	γ	γ
Elberta peach................	Chlorotic	8	9.31	26.24	0.144
Elberta peach................	Green	8	20.80	52.86	0.349
Bartlett pear.................	Chlorotic	3	6.81	16.57	0.109
Bartlett pear.................	Green	3	21.33	38.50	0.350
Jonathan apple...............	Chlorotic	1	4.00	14.5	0.077
Jonathan apple...............	Green	1	15.00	39.8	0.268
Concord grape................	Chlorotic	1	4.90	19.0	0.078
Concord grape................	Green	1	12.30	36.0	0.187
Prune.......................	Chlorotic	1	6.50	25.0	0.123
Prune.......................	Green	1	20.5	51.0	0.410
Mean......................	Chlorotic		7.88	22.73	0.126
Mean......................	Green		19.87	47.51	0.336
Mean difference......................................			11.99**	24.78**	0.210**

** Mean difference significant beyond the 1 per cent level.

TABLE 7

Iron extracted from chlorotic and green grape leaves by various acids

Extractions made in absence of air

| EXTRACTING SOLUTION | CHLOROTIC LEAVES | | | GREEN LEAVES | | |
	Fe^{++}	Fe^{+++}	Total	Fe^{++}	Fe^{+++}	Total
	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$
N HCl......................	19	36	55	36	25	61
1.45 N HAc.................	106	0	106	124	0	124
1.14 N HCOOH.............	33	14	47	53	12	65

starch. All acids extracted greater amounts of ferrous iron from green than from chlorotic leaves. This favors the assumption that the differences in extractable ferrous iron are not entirely due to a larger proportion of active organic matter in the green leaves. This assumption is further supported by the relatively large quantity of ferrous iron extracted from the leaves by acetic acid and the low re-

ducing power of this acid in the presence of either glucose or starch. It is also notable that in all cases greater quantities of ferrous iron were obtained from the

TABLE 8
Reduction of ferric iron by various chemicals as influenced by glucose and starch
250 γ ferric iron present

	Fe^{++} AFTER ½ HOUR	Fe^{++} AFTER 1 HOUR
	γ	γ
Glucose + 1 N HCl	12.0	18.5
Glucose + 3% AlCl$_3$	13.0	26.5
Glucose + 1.45 N HAc	5.0	13.5
Glucose + 1.14 N HCOOH	13.5	30.0
Starch + 1 N HCl	8.5	13.0
Starch + 3% AlCl$_3$	6.5	13.0
Starch + 1.45 N HAc	1.5	6.5
Starch + 1.14 N HCOOH	18.0	40.0
1.45 N HAc alone*	0.5	0.5
CO$_2$, 1 atmosphere pressure	0.0	0.0

* No appreciable reduction of ferric iron by any of the chemicals alone.

TABLE 9
Oxidation, reduction, and retention of iron added to leaf extracts
250 γ ferric iron added per 50 ml. of suspension containing 2 gm. of green leaf tissue

PLANT	LEAF APPEARANCE	LEAVES GROUND IN N HCl		LEAVES GROUND IN WATER	
		Fe^{+++} reduced	Fe retained	Fe^{+++} reduced	Fe retained
		γ	γ	γ	γ
Grape	Chlorotic	91	153	10	75
Grape	Green	129	93	245	5
Peach	Chlorotic	51	0	0	125
Peach	Green	107	50	0	105
Apple	Chlorotic	151	41	0	250
Apple	Green	196	84	0	250
Pear	Chlorotic	71	218	150	65
Pear	Green	87	160	175	57
Prune	Chlorotic	69	72		
Prune	Green	79	43		
Mean	Chlorotic	87	97		
Mean	Green	120	86		

green leaves whereas the opposite tendency is observed for ferric iron. It should be pointed out, however, that green leaves usually contain larger quantities of sugars than chlorotic leaves. This might have increased iron reduction by the reagents.

If green leaves maintain a greater proportion of iron in reduced form than do chlorotic leaves, the oxidizing or reducing potential in reference to iron should be indicated by adding solutions of ferrous or ferric iron to freshly ground leaf tissues. In one investigation 2-gm. samples of fresh leaves were ground in the presence of 47.5 ml. of either N HCl or boiled distilled water. To each ground sample $2\frac{1}{2}$ ml. of solution containing 250 γ of iron as ferric chloride or 110 γ as ferrous chloride was added. After 30 minutes, aliquots were taken for analysis. The results are shown in table 9. "Iron retained" is that which will not react with the dipyridyl reagent with or without a reducing agent. In all cases extracts of green leaves reduced more ferric iron than the extracts of chlorotic leaves. In no case was any of the added ferrous iron retained or oxidized by the leaf extracts, and therefore these results are not tabulated.

TABLE 10

Oxidation, reduction, and retention of iron by expressed leaf sap

250 γ Fe^{+++} and 110 γ Fe^{++} added to 1 ml. sap

PLANT	LEAF APPEARANCE	pH OF SAP	250 γ Fe^{+++} ADDED		110 γ Fe^{++} ADDED	
			Fe^{+++} reduced	Fe retained	Fe^{++} oxidized	Fe retained
			γ	γ	γ	γ
Concord grape.......	Chlorotic	3.57	240	10	0	20
Concord grape.......	Nonchlorotic	3.55	250	0	0	6
Pear................	Chlorotic	5.89	130	30	40	20
Pear................	Nonchlorotic	5.52	184	50	20	20
Peach...............	Chlorotic	5.56	188	20	0	0
Peach...............	Nonchlorotic	5.42	220	20	0	0
Vinifera grape.......		3.02	224	26	0	0

In table 10 are reported representative results of similar tests in which expressed plant sap was used in place of the acid or water extracts. One milligram of the expressed sap was added to make a total volume of 25 ml. containing either the ferrous or ferric iron. The sap exhibited somewhat greater reducing powers than the extracts, but the tendency for green leaves to reduce iron more rapidly than the chlorotic leaves is again demonstrated.

Some preliminary measurements were obtained on oxidation-reduction potentials showing that, in general, the saps from chlorotic leaves have more negative potentials than those from green leaves. Further studies are being conducted on potentials of both soils and plant tissues.

DISCUSSION

The data obtained support the concept that ferrous iron is of fundamental importance in plant nutrition and chlorophyll formation. Results of this study and theoretical considerations indicate that no appreciable quantities of ferrous iron

would exist for any period of time in high-lime soils under common moisture conditions. A reaction taking place between ferrous iron and soil lime can be represented as follows:

$$Fe^{++} + CaCO_3 = FeCO_3 + Ca^{++}$$
$$4FeCO_3 + O_2 + Ca(HCO_3)_2 = 2Fe_2(CO_3)_3 + Ca(OH)_2$$
$$Fe_2(CO_3)_3 + 3H_2O = Fe_2O_3 + 3H_2CO_3$$
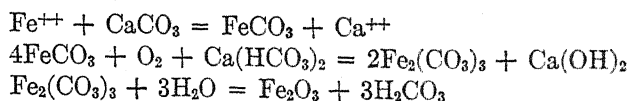
The second reaction is reported to proceed vigorously with release of considerable heat when ferrous carbonate is in a moist condition (18, p. 363). Since ferrous iron is relatively unstable in the presence of lime, its existence at the root-hair surface would depend on how easily iron in the soil can be reduced and on the reducing potential exerted by plant root and local soil components. Forms in which iron occurs and the general oxidation-reduction potential of the soil would also be significant factors in controlling iron availability in ferrous form.

Since the acid extracting reagents employed are able to reduce ferric iron in the presence of organic matter, an accurate estimate of the quantity of ferrous and ferric iron in soils is not possible. The reduction of ferric iron by aluminum chloride solution in the presence of glucose and starch raises the question whether ferrous iron extracted from soils by Ignatieff (15) represented ferrous iron naturally present in the soil, or whether it was in part that reduced by the reagent.

In the present study no determinable quantities of iron were removed from the soils by the ammonium acetate solution at pH 5 without hydroquinone. It is concluded, therefore, that the quantities of soluble and replaceable ferrous or ferric iron present in the original soil samples were too small to be of practical significance in plant nutrition. If iron is assimilated in ferrous form reduction would have to take place near the point of assimilation.

Sherman et al. (26, 27) concluded that manganese reduced by a solution of 0.2 per cent hydroquinone at pH 7 is a measure of active manganese available for plant nutrition. According to their standard, by which 100 p.p.m. of easily reducible manganese is necessary to supply plant needs, all of the soils studied here must be deficient in this element. Since plant deficiency symptoms are not noticeable on many of these soils, the standards established for calcareous soils in Kentucky must not be directly applicable to calcareous soils of this area.

The value of reducing solutions for estimating iron availability in calcareous soils needs further study, but in this investigation iron has been more easily reduced in soils producing green plants than in those producing chlorotic plants.

Though the significance of ferrous iron within the plant seems to be widely accepted, little is known concerning internal plant factors associated with ferrous-ferric iron equilibrium. The difference in capacity of the sap of chlorotic and green leaves to reduce ferric iron indicates a difference in the oxidation-reduction systems within the leaves. Relations between pH and oxidation potential cannot be ignored, but since pH in itself has not always differed between comparable samples of chlorotic and green leaves, a combined consideration of pH and Eh may furnish a more complete explanation of iron mobility and chlorophyll formation.

Available information does not suggest any direct relation between the calcium, potassium, and nitrogen content of leaves and the state of oxidation, but the influence of these elements on synthetic processes indicates that under given conditions they might favor more oxidizing or reducing potentials.

This investigation does not solve the problem of whether chlorosis is due to iron unavailability in the soil or to lack of mobility in the plant, but the study does suggest that the same relationships regarding ferrous and ferric iron exist in both plant and soil. It is probable that the effects are additive in bringing about iron immobility.

SUMMARY

In a study of soil samples from areas producing chlorotic plants in comparison with samples from nearby areas producing green plants it was found that the latter soils contained significantly more readily reducible iron and manganese (by 0.2 per cent hydroquinone solution) than did soils producing chlorotic plants. Ferrous and ferric iron salts added to either class of soil were quickly immobilized, only small quantities remaining reducible by 0.2 per cent hydroquinone.

Chlorotic leaves from peach, pear, grape, prune, and apple contained more potassium and nitrogen and less iron and calcium than did green leaves.

The iron content of green leaves was significantly higher than that of chlorotic leaves when results were expressed on the basis of leaf area, but when expressed on a dry weight basis the mean difference was not significant. Similar relations were also found for the manganese and calcium content of leaves with respect to method of expressing the results.

No significant difference was found in the chemical composition of recent terminal branch growth from chlorotic and green trees. Iron content of fruit from peach and pear trees was appreciably greater for green than for chlorotic trees.

Green leaves contained more iron soluble in N HCl than did chlorotic leaves. Hydrochloric, acetic, and formic acid solutions each extracted appreciably more ferrous iron from green leaves than from chlorotic leaves. Although these acids are able to reduce some ferric iron in the presence of glucose and starch, the results are considered sufficiently distinct to indicate a greater quantity of ferrous iron in the green leaves.

Extracts and sap from green leaves had a greater capacity to reduce ferric iron than did similarly obtained extracts and sap from chlorotic leaves.

The results obtained indicate that soil and plant conditions associated with chlorosis are more conducive to the maintenance of iron in insoluble ferric compounds than are conditions in both soils and plants associated with normal green leaf development.

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THE TIRS OF MOROCCO

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Half a centrury ago the black soils of Morocco and of southern Spain were mentioned in scientific literature, but it is only recently that they have been studied by pedological methods.

The black Spanish soils have been discussed to some extent in the book *Soils of the Lusitano-Iberian Peninsula*[2], where complete analyses of two typical profiles are reported. Our knowledge of the tirs of Morocco is based on a study of more than 200 profiles. These were made on freshly exposed cuts and were carefully sampled and analyzed. This paper is a brief résumé of a detailed study to appear under the title "Soil Types of North Africa."

"Tirs" is a Berberian term which in Tashelhit dialect signifies "soil" and in Tamazirt, "humus," and is now applied by the natives of Morocco to soils that appear to contain much humus.[3] There are several subtypes of tirs, such as *glei tirs* and *crust tirs*, which may be considered as distinct soil types. Soils containing mixed features of both these types are also found. A gray to light gray soil, called "fershesh" by the natives, and two other kinds of black soil— *deep tirs* and *broken-land tirs*—are related to the glei tirs.

The typical glei tirs, of which there are two varieties, black and gray, extend through northwest Morocco (Gharb) on the low flat plains drained by the Sebou, the Lukkus, and smaller rivers and wadis that flow to the Atlantic Ocean. The climate is southern Mediterranean, with hot, dry summers and mild, rainy winters. The rainfall ranges from about 500 or 600 mm. near Port Lyautey to about 800 between Arzila and Tangier.

Along the main rivers of the low, flat plain lie strips of alluvial soils of varying widths, called *dess* by the natives. The glei tirs occupy the large depressions between the streams. The black tirs is found in the lower microrelief levels of the depressions, and the gray tirs occupies the other levels. The tirs generally have a much higher water table than the dess.

Many areas of the low tirs plain become waterlogged under the heavy winter rains and occasional floods, which also raise the water table. In many cases this condition is of such long duration each year that the land becomes a more

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[1] EDITOR'S NOTE: This paper, translated from the Spanish by the author himself, was extensively revised by the editors with the assistance of Jacob S. Joffe, to whom grateful acknowledgment is made. The meaning of some passages in the original version was not entirely clear, and as neither the edited manuscript nor the proof was seen by the author, responsibility for any misinterpretation of these passages rests with the editors.

[2] Emilio H. del Villar (Trans. by G. W. Robinson) 1937 Soils of the Lusitano-Iberian Peninsula. Thomas Murby & Co., London.

[3] As summarized by Joffe (Joffe, Jacob S. Pedology, p. 222. Rutgers University Press, New Brunswick, N. J., 1936), "tirs" is "ethnologically a Berberian name meaning 'black humus earth.' This name is also known in Algeria where it signifies a heavy clay soil."

or less permanent marsh (*merdja*). Consistent drainage work by the French
farmers has resulted in a gradual reduction in the extent of the merdjas.

The natural vegetation of the flooded tirs consists of an association of
Phragmites communis with *Juncus acutus* and *J. maritimus* and of *P. communis*
with *Scirpus maritimus* (the *diss* of the natives). The latter tends to become
dominant when the soil is still waterlogged but not submerged. In the course
of the drying process the succession of species is, first, a moist prairie of Gram-
inaceae and Papilionaceae, and finally a carpet of xerophytic short grass and
herbaceous species. On certain reclaimed tracts of gray and of deep black tirs,
this carpet becomes a thick savanna of tall grasses and herbs, mainly Umbel-
liferae, such as *Ridolfia segetum* and *Daucus maximus*, and thorny composites,
such as *Scolymus maculatus*, reaching a height of more than 2.5 m.

The areas of glei tirs and dess are surrounded, to the west and south, by red
sandy soils, formed on the downs or on materials of similar origin. To the east,
the areas border isolated tracts of the same downs and hills and low mountains,
where the broken-land tirs is dominant. Some transverse offshoots of these
two formations divide the low flat area into several separate basins, the largest
of which—the southern—is crossed by the low Sebou and the Wadis Madegh
and Mda-Segmet. It was in this basin that most of the glei and glei profiles
were studied.

The natural vegetation of the mature or secondary red sandy soils is a forest of
Quercus suber with scattered groups of *Pyrus mamorensis*, this latter having
grown also, it appears, on the immature sandy soils. The suberetum includes
a woody and herbaceous undergrowth containing many calcifuge species. Scat-
tered remnants of this forest still exist, but the only extensive area remaining is
the great tract of La Mamora, south of the low Sebou plain.

It is presumed that the black tirs is formed from the red sandy soil, and the
gray tirs, from the dess, by the same hydro-hypogenic process.

THE GLEI TIRS

Essentially, a glei tirs profile consists of the following horizons:

1. One or more upper horizons of heavy clay, with columnar macrostructure and cloddy-
platy-granular cleavage. The granules are to be found mainly at the tilled surface.
2. One or more lower mottled glei horizons, which may be clayey or sandy, never loamy
or silty, and which typically have a polyhedral structure.
3. Sandy or sandy-clay horizons, which may be interposed between or occur below the
columnar and the glei horizons. These are the remains of the red sandy parent soils.

The water table is never far from the surface, its usual depth being less than
2 m. The ground water and generally the lower horizons are saline, but the
upper horizons normally are relatively free from quantities of salts injurious to
vegetation. Plant residues are found chiefly in the upper horizons but are not
necessarily abundant. Mollusk shells or their remains usually are present and
at times are abundant in almost all horizons, as are old root and worm holes.

The glei horizons correspond to the depth at which the changing ground-water
level causes the most frequent alternations of aerobic and anaerobic conditions,

and therefore of oxidation and reduction. This results in a diversity of products, such as calcareous "bieloglazka" and white saline efflorescences, and in mottling, which produces yellowish, brown, rusty, reddish, greenish, bluish (vivianite), and black patches.

The columnar macrostructure of the upper clay horizons is very different from that of solonetz, being coarser and less regular. When the clayey mass is water-logged, the profile face appears massive and amorphous. Upon drying, how-ever, it soon cracks vertically. These fissures are marked at the surface by a polygonal network, but with depth, they become narrow and branch out. Aside from this vertical macrostructure, the mass breaks into clods and lumps, and these into platy chips, which in turn break into irregular granules—a process from which is derived the term "cloddy-platy-granular" structure. The material varies in consistency, depending on conditions: when waterlogged it is soft and smeary; on drying, it soon becomes so hard that it can be broken only with a hammer. These mechanical features are common to the black and gray tirs. In table 1 the mechanical composition of the clayey horizons of black[4] and gray glei tirs is given. In table 2 the average mechanical composition of many samples of various materials and types of tirs is given.

Analyses of black tirs do not seem to bear out the assertion of some authors that these soils are high in organic matter. More organic matter has been found in the soils intermediate between the red sands and the black tirs than in the black tirs itself. This is especially true for the virgin soils of the black sands, which are discussed presently. There seems to be a gradual loss of organic mat-ter as the black tirs dries up. Among 38 profiles of black glei tirs examined, the organic matter content varied from 0.589 to 2.649 per cent in the surface horizon. More than 75 per cent of the samples had an organic matter content above 1.0 per cent. Data on the organic matter content of several types of tirs are given in table 3.

The black glei tirs, as a rule, contain carbonates, but they are not pedocals. The depth at which the carbonate is found varies in different profiles, and the increase in carbonate is not constant with depth. The carbonate is the result of upward movement of salts. The parent material, the red sand, is devoid of carbonates. The "bieloglazki" in the profiles are the remnants of mollusk skeletons.

Soluble sulfates are generally found in these soils, usually in the deep levels, though small quantities are sometimes found in the upper levels of the profile.

Chlorides are more frequently found, usually in the lower horizons and some-times in the upper horizons. In terms of NaCl, less than 0.03 per cent was found in the surface of 51.1 per cent of the profiles examined. In the deeper layers the NaCl content rises, being within the limits of 0.03 and 0.3 per cent.

The HCl extracts of these soils shows some interesting features. There is as much as 30 per cent colloidal SiO_2. The R_2O_3 content is also high, indicating an abundance of sialferic complex. The SiO_2/R_2O_3 ratio varies but does not

[4] In 19 of 45 profiles of black glei tirs examined, almost all during the dry season, the water table was at a depth of less than 2 m.

reach 4; the SiO_2/Al_2O_3 does not go higher than 4 and a fraction; and the SiO_2/Fe_2O_3 goes up to 7, although in one case it was found to be 11.91. The data on these and other chemical values are given in tables 4 and 6.

An analysis of the absorbing complex is given in table 7. It shows an abundance of colloids and a state of base saturation because of the upward movement

TABLE 1

Mechanical composition of the glei clayey horizons of black and gray tirs

	ATTERBERG'S FRACTIONS, PERCENTAGE OF THE TOTAL MECHANICAL FRACTION				TOTAL MECHANICAL FRACTION, PERCENTAGE OF THE 2-MM. DRY SAMPLE
	I ($<2\mu$)	II ($2-20\mu$)	III ($20-200\mu$)	IV ($200\mu-2$ mm.)	
*Black tirs**					
Average....................	73.10	16.26	8.42	0.40	.....
Minimum...................	60.53	8.16	0.10	0	53.46
Maximum...................	91.26	28.04	23.59	2.67	96.77
Gray tirs†					
Average....................	75.58	22.44	1.92	0.06	.....
Minimum...................	59.61	15.32	0.06	0	79.44
Maximum...................	84.62	39.26	7.28	0.21	87.29

* Analysis of 55 horizons from 16 profiles.
† Analysis of 14 horizons from 3 profiles.

TABLE 2

Average* mechanical composition of many samples of various materials and types of tirs

SOIL TYPES	ATTERBERG'S FRACTIONS, PERCENTAGE OF THE TOTAL MECHANICAL FRACTION			
	I ($<2\mu$)	II ($2-20\mu$)	III ($20-200\mu$)	IV ($200\mu-2$ mm.)
Red sandy soils of the downs.........	14.53	5.11	77.99	2.36
Muck underlain with black sands....	25.05	4.55	67.51	2.89
Transitional from black sands to black glei tirs.....................	40.95	14.60	36.73	7.72
Black glei tirs (clayey horizons).....	73.10	16.26	8.42	0.40
Gray glei tirs........................	75.58	22.44	1.92	0.06
Dess...............................	47.03	33.76	19.20	0.00

* These averages refer only to the typical horizons of each soil type: the red and sandy horizons of the downs; the black and sandy horizons of the black sands; the tirsified horizons of the tirs; and all horizons of the dess, the profiles of which are very nearly uniform.

of salts. There is very little sodium in the absorption complex. These soils are therefore not of the alkali type[5]. The pH values of the black glei tirs vary.

[5] In accordance with the nomenclature of the Second Commission of the International Soil Science Society, the term *saline* is applied to soils containing an appreciable quantity of soluble salts; and *alkali*, to those that contain appreciable alkali in the complex.

In 81 per cent of the 164 horizons examined, the pH fluctuated between 7.3 and 7.7; the lowest pH value was 6.4.

TABLE 3

Organic matter content of different types of tirs of Morocco

Percentage of the 2-mm. sample dried at 105°C.

PROFILES AND HORIZONS	HUMUS*	CARBON	ORGANIC MATTER (C × 1.724)	NITROGEN	C/N	HUMUS/C	HUMUS × 100 / ORGANIC MATTER	N × 100 / ORGANIC MATTER	N × 100 / HUMUS
	per cent	*per cent*	*per cent*	*per cent*					
Black glei tirs†									
Gh-107-I.........	2.305	1.990	3.431	0.221	9.01	1.16	67.18	6.44	9.58
II.......	1.457	1.258	2.168	0.098	12.88	1.16	67.18	4.50	6.70
III.......	1.323	1.159	1.998	0.090	12.82	1.14	60.20	4.52	6.83
Gh-104-I.........	1.578	1.523	2.625	0.146	10.44	1.03	60.11	5.55	9.24
II.......	1.340	1.034	1.783	0.114	9.06	1.29	75.75	6.40	8.52
III.......	0.539	0.663	1.142	0.060	11.47	0.81	47.21	5.21	11.03
Gray glei tirs‡									
Gh-121-I.........	0.898	0.745	1.284	0.087	8.60	1.20	69.88	6.74	9.65
Crust tirs§									
Cas-7-I...........	2.049	1.423	2.453	n.d.	.....	1.44	83.52	....	....
Cas-8-I...........	1.821	1.224	2.110	0.111	11.07	1.49	86.30	5.24	6.02
II.........	1.257	0.881	1.518	n.d.	.....	1.43	82.78	....	....

* "Humus" includes the organic material extracted by 1 per cent NaOH from the decalcified sample. Analyses by the author.

† Gh-107. French Gharb from Kudiat Sba, north of the Mda-Segmet Canal, saline water table at a depth of 85 cm., forming the lower boundary of horizon III. Sample taken on November 27, 1937, from a field in cereal grains. Horizon I, 31 cm. thick; black, clayey; columnar macrostructure and cloddy-platy-granular breaking. Horizon II, 35 cm. thick; black with some white spots; clayey; columnar macrostructure. Horizon III, 19 cm. thick; black with gray-blue tinges and small white spots; subpolyhedral structure; glei-like.

Gh-104. French Gharb from Kudiat Sba, south of the Wadi Mda Canal, saline water table at a depth of 165 cm. Sample taken on November 26, 1937, from cultivated soil. Horizon I, 58 cm. thick; black, clayey; the structure and the breaking like those of Gh-107-I and -II, when slightly dry, with a network of polygonal cracks at the surface. Horizon II, 64 cm. thick; blackish or black; clayey and columnar. Horizon III, 43 cm., extending down to the water table; glei horizon; greenish gray, becoming mottled with gray-white on drying; clayey, with polyhedral structure (subpolyhedral when very moist).

‡ Gh-121-I. Surface horizon of a profile 5 to 6 m. deep sampled in 1937 at the Bled-Marktan, near the Abd-er-Rahman Canal, then under construction; selected because this tract had just been drained and was of the darkest shade recorded among the gray glei tirs.

§ For profile description, see table 9.

The physical and chemical analyses and the properties of the absorbing complex show that gray and black glei tirs differ only in degree of development. The gray tirs, which forms from dess, has a rather uniform profile. Unlike the

TABLE 4

Chemical analysis* of the hydrochloric acid extract of the black glei tirs profile Gh-107†

PERCENTAGE COMPOSITION BY WEIGHT OF THE 2-MM. SAMPLE DRIED AT 105°C.				CATIONS			
	I (0–31 cm.)	II (31–66 cm.)	III (66–85 + cm.)		I (0–31 cm.)	II (31–66 cm.)	III (66–85 + cm.)
Na_2O	0.75	1.05	0.94	Total.............. m.e./100 gm.	1,308.63	1,362.70	1,348.56
K_2O	1.07	0.87	0.84	Percentage of the cations:			
MgO	1.56	2.00	2.03				
CaO	8.79	7.00	6.18	Na^+	1.85	2.49	2.26
MnO	0.42	0.29	0.20	K^+	1.74	1.36	1.33
Fe_2O_3	6.70	6.88	6.95	Mg^{++}	5.92	7.29	7.47
Al_2O_3	10.34	11.82	12.13	Ca^{++}	23.96	18.32	16.35
SO_3	0.18	0.25	0.37	Mn^{++}	0.92	0.61	0.43
P_2O_5	0.19	0.19	0.21				
CO_2	3.60	3.74	3.48	Total monovalent and divalent	34.39	30.07	27.84
SiO_2	18.54	30.83	29.56	Fe^{+++}	19.23	18.98	19.36
TiO_2	n.d.	n.d.	n.d.	Al^{+++}	46.38	50.95	52.80
Insoluble residue	38.30	25.29	27.38				
Loss on ignition	10.60	9.69	8.68	Total trivalent	65.61	69.93	72.16
Total	101.04	100.53	99.05	Ratio monovalent and divalent/ trivalent	0.52	0.43	0.38
Fine fraction	51.10	64.39	63.94				
Sialferic complex, per cent of fine fraction	70.00	77.22	76.40				

* International method. Analysis by Ing. N. Preisig, in the De 'Sigmond Laboratory, now under Dr. Kotzmann's direction, Royal Joszef Institute, Budapest. For SiO_2/R_2O_3 see table 6.

† For profile description, see table 3.

TABLE 5

Chemical analysis* of the hydrochloric acid extract of the gray glei tirs profile Gh-18†

PERCENTAGE COMPOSITION BY WEIGHT OF THE 2-MM. SAMPLE DRIED AT 105°C.					CATIONS			
	I (0–9 cm.)	II (9–37 cm.)	III (37–112 cm.)	IV (112–152 cm.)		I (0–9 cm.)	II (9–37 cm.)	III (37–112 cm.)	IV (112–152 cm.)
Na_2O	0.71	0.57	0.50	0.66	Total...... m.e./100 gm.	1,136.10	1,122.11	1,150.58	1,174.19
K_2O	0.86	0.84	0.77	0.64	Percentage of the cations:				
MgO	1.19	1.66	1.11	1.90	Na^+	2.02	1.63	1.40	1.81
CaO	9.77	9.70	9.90	7.37	K^+	1.62	1.59	1.42	1.16
MnO	0.43	0.18	0.31	0.27	Mg^{++}	5.22	7.32	4.77	8.05
Fe_2O_3	6.45	6.39	6.28	6.40	Ca^{++}	30.67	30.82	30.69	22.39
Al_2O_3	7.35	7.02	7.92	9.09	Mn^{++}	1.06	0.46	0.77	0.66
SO_3	0.12	0.13	0.10	0.17					
P_2O_5	0.17	0.18	0.18	0.16					
CO_2	6.27	5.90	6.33	3.76	Total monovalent and divalent	40.59	41.82	39.05	34.07
SiO_2	15.24	13.96	15.08	17.17	Fe^{+++}	21.42	21.43	20.53	20.47
TiO_2	n.d.	n.d.	n.d.	n.d.	Al^{+++}	37.99	36.75	40.42	45.46
Insoluble residue	44.38	46.60	44.96	45.78					
Loss on ignition	8.04	8.06	7.22	7.56	Total trivalent	59.41	58.18	60.95	65.93
Total	100.98	101.19	100.66	100.93	Ratio monovalent and divalent/trivalent	0.68	0.72	0.64	0.51
Fine fraction	47.58	45.34	47.82	46.66					
Sialferic complex, per cent of fine fraction	61.39	60.76	61.60	71.34					

* International method. Analysis by Ing. N. Preisig. For SiO_2/R_2O_3 see table 6.

† French Gharb, low basin of the Sebou, northwest of Et-Tleta du Gharb; water table at a depth of 160 cm.; sample from field in soybeans. Horizon I, 8 to 9 cm. thick; granular structure; medium gray; polygonal network of cracks. Horizon II, 26 to 30 cm. thick; medium gray, clayey, with very regular typical macrostructure. Horizon III, 70 to 80 cm. thick, medium to dark gray, clayey, with less marked columnar structure. Horizon IV, 40 cm. thick; black (ancient black tirs horizon), clayey, showing a transitional structure, from cloddy-platy-granular (the breaking type of the upper tirs horizons) to polyhedral (typical of the glei). Horizon V, below 150 cm., affected by the water table; typical glei, yellowish gray, clayey, polyhedral (imperfect structure when waterlogged). Ground water contains 1.21 per cent NaCl.

black tirs, the gray tirs is not related to the sands. The water table has been found at a depth of more than 2 m. in two-thirds of the gray tirs profiles studied, and at less than 2 m. in the remainder.

TABLE 6

Comparison of certain characteristics of the hydrochloric acid extracts of red sandy soil, black and gray glei tirs, and dess

SOIL TYPE	HORIZON	FINE FRACTION, % OF 2-MM. DRIED SAMPLE	SIALFERIC COMPLEX, % OF FINE FRACTION	CATIONS, M.E. PER 100 GM.	CATIONIC RATIO, MONOVALENT AND DIVALENT/TRIVALENT	MOLECULAR RATIOS			
						SiO_2/R_2O_3	SiO_2/Al_2O_3	SiO_2/Fe_2O_3	Al_2O_3/Fe_2O_3
Alg-85*, red sandy soil of the downs	Present A horizon (0–30 cm.)	43.20	20.18	931.77	2.70	1.05	3.34	1.53	0.45
	1st fossil B horizon (80–110 cm.)	10.37	87.08	300.34	0.13	1.10	2.83	1.80	0.64
	2d fossil B horizon (280–305 cm.)	13.23	73.47	404.00	0.36	0.77	1.58	1.48	0.93
Gh-107†, black glei tirs	I (0–31 cm.)	51.10	70.00	1,308.63	0.52	2.15	3.04	7.35	2.41
	II (31–66 cm.)	64.39	77.22	1,362.70	0.43	3.23	4.41	11.91	2.69
	III (66–85 + cm.)	63.94	76.40	1,348.56	0.38	3.03	4.10	10.86	2.73
Gh-18‡, gray glei tirs	I (0–9 cm.)	47.58	61.39	1,136.10	0.62	2.26	3.51	6.28	1.78
	II (9–37 cm.)	45.34	60.76	1,122.11	0.72	2.13	3.37	5.81	1.72
	III (37–112 cm.)	47.82	61.60	1,150.58	0.64	2.15	3.22	6.39	1.98
	IV (112–152 cm.)	46.66	71.34	1,174.19	0.51	2.21	3.20	7.12	2.22
Gh-87§, recent dess	I (0–44 cm.)	41.50	42.77	1,126.50	1.21	1.71	2.85	4.15	1.43
	II (44–117 cm.)	42.18	40.02	1,035.11	1.33	1.66	2.80	4.04	1.43
	III (117–172 + cm.)	40.80	33.26	1,020.10	1.74	1.44	2.66	3.13	1.17

* Profile Alg-85 (from the Algerian Sahel, between Guyotville and Staouéli) shows three soils one above the other: the present, not yet decalcified; the two fossil soils with crusts or hardpans and with B and C but without A horizons. For these special conditions, this profile was selected in preference to any of the Gharb profiles.

† See table 3.

‡ See table 5.

§ French Gharb from the left bank of the Sebou, north of Mograne; natural associations of *Vitex-agnus-castus* (typical on the recent dess) with *Tamarix* near the flow, and plantings of *Populus*; water table at a depth of 7 m. Horizon I: light to medium gray, tinged with yellow; clayey-silty texture; cloddy-platy-granular structure with no columnar cracking throughout the profile and no network of fissures at the surface. Horizon II, light gray, tinged with yellow; silty-clayey texture and subpolyhedral structure. Horizon III, light to medium gray; sandy texture and polyhedral structure.

From the analyses made thus far, it appears that the amount of humus in gray tirs is usually less than that in the black tirs, but, as shown in table 3, the characteristics of the organic matter are similar in both these soils.

As in the black tirs, the carbonates indicate no dominant leaching process. The lime in the profile of the gray tirs, however, is due not only to the upward

movement of salts and to the presence of mollusk shells, but also to the parent material, which is calcareous. The distribution of carbonates is therefore more uniform in the profile of the gray tirs than in the black.

TABLE 7

Analysis of absorbing complex and water-soluble bases of the black glei tirs profile Gh-107

		I (CaCO$_3$ = 8.22%)	II (CaCO$_3$ = 8.54%)	III (CaCO$_3$ = 7.91%)
Water-soluble bases, % of 2-mm. sample dried at 150°C.	Na$_2$O..........	0.006	0.105	0.107
	K$_2$O..........	0.006	0.003	0.003
	MgO..........	0.022	0.032	0.024
	CaO..........	0.673	0.516	0.426
Percentage equivalents of dissolved cations	Na$^+$..........	0.78	14.39	17.30
	K$^+$..........	0.49	0.29	0.37
	Mg^{++}..........	4.33	6.75	5.88
	Ca^{++}..........	94.40	78.57	76.45
Exchangeable cations, m.e. per 100 gm.	Na$^+$..........	1.89	2.04	2.09
	K$^+$..........	0.60	0.40	0.41
	Mg^{++}..........	3.94	9.30	6.60
	Ca^{++}..........	44.00	44.80	63.20
S (Sum of absorbed cations).....................		50.43	56.54	72.30
T (Absorption capacity).........................		53.03	59.44	75.50
T − S..		2.6	2.9	3.2
V (Degree of saturation).......................		95.09	95.12	95.76
Equivalents, as percentages of T	Na$^+$..........	3.57	3.43	2.77
	K$^+$..........	1.14	0.68	0.54
	Mg^{++}..........	7.43	15.11	8.74
	Ca^{++}..........	82.96	75.54	83.71
	H$^+$..........	4.90	5.24	4.24

Comparative calculations—percentage by weight of the 2-mm. dried sample

	I	II	III
Soluble Na....................................	0.005	0.078	0.079
Exchangeable Na...............................	0.044	0.047	0.048
Total Na removed..............................	0.048	0.124	0.127
NaCl corresponding theoretically to this total....	0.123	0.317	0.324
Soluble K.....................................	0.005	0.003	0.003
Exchangeable K................................	0.024	0.016	0.016
Total K removed...............................	0.029	0.018	0.019
K$_2$O corresponding to this total..................	0.035	0.022	0.023

Variable amounts of sulfates are found only in the ground waters or in the glei or glei-like horizons of the gray tirs. In 78 per cent of the profiles, sulfates were found in very small quantities.

As for the chlorides, the results of the analysis of numerous gray tirs samples are as follows: at the surface, 0.03 per cent in 67 per cent of the profiles and 0.03

to 0.3 per cent in the other 33 per cent; in the profile as a whole, 0.03 per cen in 5.5 per cent, 0.03 to 0.3 per cent in 50 per cent, and more than 0.3 per cen in 44.5 per cent. The maximum chloride content of the gray tirs is not so high as that of the black tirs.

In table 6 are presented data on the various types of tirs. It is clear that a the process of development goes from the black tirs to the dess, through the inter mediate gray tirs, (see also tables 4 and 5), the following changes take place 1, loss of the fine fraction and consequent reduction in the amount of colloids 2, loss in absolute weight of the sialferic complex and a change in the ratio of this to the fine fraction; 3, loss of colloidal silica in relation to sesquioxides; 4, loss o alumina in relation to the iron oxide; 5, increase in percentage of bases. That gray and black tirs are more closely related than are the gray tirs and the dess is evidenced by the ratio of the sialferic complex to the fine fraction and by the ratio of monovalent plus divalent to trivalent cations (always < 1). Both tirs varieties are calciferous but with a sialferoid composition, whereas the dess is o basoid composition.

The pH values of the gray tirs vary from 7.2 to 7.65. They exceed 7.5 in only 4.2 per cent of the determinations made, and are less than 7.3 in only 5.3 per cent of the determinations; in the main they range between 7.3 and 7.5.

To appreciate the process of glei tirs formation, one must remember that the surface of the earth is affected, not only epigenically by climatic factors, but also hypogenically by a system of other fundamental factors. This system is of plutonic nature. To it may be referred the origin of thermal springs, metallic complexes, and volcanic phenomena from the smallest mud volcanoes (Spanish *salsas*), like those of Andalusia[6], to the highest conical mountains emitting vast lava flows. In many craters, when the eruptive activity ceases, salt lakes are formed. In other cases the plutonic activity is reduced to emissions of thermal waters or gaseous materials. Still, many of the materials brought in by inactive volcanoes and by the little "salsas" are of the same nature as those which appear in the salt lakes and are similar to the other products of the saline soil-forming process: thermal water, soluble silica, black muds with sulfur bacteria, soluble salts, and gypsum.

The transformation of the red sands and of the alluvial loams into glei tirs belongs to the same cycle of phenomena. The low flat plains of the tirs are depression areas similar to the areas of saline patches throughout the world. In both cases, the depression is not the cause of the hydro-hypogenic process, but the effect.

The successive stages in the pedogenic process of transformation from the red sandy soil to the black glei tirs are well known. The intermediate state is that of black clayey, sandy soil (French *sables noirs gras*). The red sands are a complex soil, chiefly epigenic, but often affected by hypogenic influences which contribute to the formation of crusts and glei horizons and which bring in some saline materials. Around the glei tirs depressions, the red sandy soils are for the

[6] See footnote 2.

most part decalcified, showing in their upper horizons an AB (or ABC) profile, but in many places their present suface corresponds to a fossil horizon and in other places to a relatively recent sand accumulation, i.e., a secondary young profile. The data in tables 2 and 6 are presented to round out the information on these soils. It is not the aim of this paper to give a full description of these soils.

The entire area of red sands around the tirs is besprinkled by patches of black sands corresponding to microdepressions. These black sands are in different stages of development. In their typical intermediate stage they are clayey fine sandy soils, containing, in their virgin state, large amounts of humus. There are also hydro-hypogenic formations of glei with hardpan at deeper levels. The water table usually is less than 2 m. deep and, at times, is very near or even at the surface. The profile is frequently noncalcareous, or sometimes very weakly calcareous, at the surface, but distinctly calcareous at the bottom. The carbonate distribution throughout each profile and among the different profiles is very irregular. This characteristic and the fact that these spots of intra-calcareous black soil appear isolated in the siallitic red sands, show that they are not a product of leaching but the result of an upward movement. The soluble salts at the surface are of a similar origin. Important, even though small, are the quantities of sulfates found in 67 per cent of the profiles, and at the surface in 55.5 per cent. The amounts of chlorides are higher: 67 per cent of the profiles contain more than 0.03 per cent of NaCl, and some of the horizons contain 0.3 per cent. The pH values vary from 7 to 7.3 in 14 per cent of the horizons studied, equal 7.3 in 32 per cent, and vary from 7.3 to 7.7 in 54 per cent.

The transition from the red sand area to that of the black sands is marked by an abrupt drop in topography. Over a distance of 5 to 7 km. one may find all the stages of the soil-forming process.

The process of transformation from the dess to the gray tirs has also been ascertained. The dess is, on the average, light yellowish-gray nearly silty clay, of rather uniform profile, never columnar, of lesser consistency than the tirs, and not so waterlogged during the rainy season. The water table is rather deep, about 4 m. in the oldest known profiles of dess and from 7 to 25 m. in those of recent formation. The dess profiles are richer in carbonates than are those of the gray tirs. These carbonates are formed from alluvial material, and the loss by leaching is more or less compensated by fresh flood deposits. The dess profiles are usually low in sulfates, and their chloride content never exceeds 0.03 per cent. Their pH values vary from 7.2 to 7.5.

The so-called "old dess," which farmers distinquish from that of recent formation, is a stage in the transition from true alluvial soil to the gray tirs. All the stages can be ascertained by examining the borders of each type and by inspecting the numerous mixed profiles in which both of these soils and perhaps the black tirs are represented.

An extensive profile of this kind was studied on a canal cut $1\frac{1}{4}$ km. long, east of the Madagh bridge on the north side of the highway to Tangier. In this exposure could be seen dess, glei tirs, and here and there one or more black

horizons at the bottom.   These last are buried tirs overlain by the dess of al-luvial origin.   The upper layers of this dess appear to have been partly and, in some instances, completely transformed into gray tirs, whereas the underlying black tirs has been changed into glei, its former columnar structure having be-come polyhedral.   In many exposed cuts the present black glei appears to have been pushed upward by the ascending hydrogenic process.   In places the glei appears in the upper layers of the dess and gray tirs; elsewhere it runs through them, sometimes to the very surface, occasionally upsetting the normal profile.

Whether the black tirs is formed from the red sands, or the gray tirs is formed from the dess, the natural process and the pedogenic product differ only in degree of development.   This soil process involves:

1. Increase of the clay fraction and loss of the silt and sand fractions.
2. A change in chemical composition, as shown by the hydrochloric acid extract data.
3. An increase in the fine fraction, which appears chiefly when the parent material consists of decalcified sands, and therefore, an increase in replaceable cations.
4. A large increase in colloidal silica, both in absolute amount and in relation to sesqui-oxides; and an absolute increase of the respective sesquioxides, chiefly of alumina.
5. An increase in the absolute amount of Na and K and in the percentage of these ions in the total replaceable cations.

In these soils we have one of the best examples of type, the pedogenesis of which is independent of the parent material.

The color of the two varieties of tirs is the result of the process of their forma-tion.   The essential feature is that both the black and the gray shades belong to the cyanic series (yellowish–greenish–bluish–black), common to soils formed under waterlogged conditions.   The humus content can be but partly responsible for the black and dark shades.   Even with a humus content of only 1 to 3 per cent, the black color may be intense, since the clayey texture and the frequent presence of Na ions result in a high dispersion of the humus.   There is no correla-tion, however, between dark color and humus content.   To show clearly the role of each factor, certain tests were carried out.   From the results, presented in table 8, the following conclusions may be drawn:

The color is markedly influenced by the degree of moisture.   Under natural conditions it is also influenced by the intensity and direction of the light.

On removal of water-soluble substances, the color becomes lighter.   This change is due to the loss of sodium ions which cause a dispersion of the humus.

On treatment with HCl, the color, mainly the black, becomes less intense.   This is prob-ably due to the loss of the sialferic complex with its black iron compounds.

Efforts to remove the humus result in darkening of the color.   The reason is that the sodium hydroxide, used for removal of the humus, leaves an excess of sodium ions which increase the dispersion of the colored substances.

Under waterlogged conditions several substances of greenish, bluish (vivianite), and black shades, chiefly iron compounds such as FeO, are formed in the soil. It is known that FeO added to Fe_2O_3 yields Fe_3O_4, which, of course, is black. The same effect is to be expected from the colloidal compounds of Fe_2O_3 and

FeO of the soil.[7] Undoubtedly the microbiological reactions also play a role in coloring the soil. This may be noted in the low-lying black soils fringing salt lakes and in the muds of "salsas," where sulfur bacteria are encountered.

Since the glei tirs derived from the red sands are black, and those derived from dess are medium to light gray, it is evident that the cause of the dark color lies in differences between the tirs varieties. Both soils belong to the same type and are the result of one and the same process. In the pedogenesis of the black tirs, however, the weathering process under waterlogged conditions has been going on much longer, and therefore the dark color is more intense. Moreover, in the black tirs the sodium ion is found more frequently and in larger quantities. This adds to the coloring of this tirs. As to the sialferic compound, there is no

TABLE 8

Results of color tests of the glei tirs

SAMPLE		Gh-107-I (BLACK TIRS, SURFACE HORIZON)	Gh-104-I (BLACK TIRS, SURFACE HORIZON)	Gh-104-III (BLACK TIRS, GLEI)	Gh-121-I (GRAY TIRS, SURFACE HORIZON)
In situ	Natural state....	Black with brown tinge	Black with brown tinge	Greenish gray (medium dark)	Grayish brown (medium dark)
Filtration residue after suspension in water for short time	Wet.......	Grayish-green-black	Grayish black	Dark greenish-yellowish gray	Dark brownish-greenish gray
	Dry......	Brown to black	Brown to black	Medium greenish-yellowish gray	Medium brownish gray
Sample decarbonated by HCl and washed to remove chloride	Wet........	Black with brown tinge	Brown to black	Dark yellowish brown	Very dark yellowish brown
	Dry.......	Medium dark gray	Medium dark gray	Medium greenish-yellowish gray	Medium brownish gray
Sample after removal of humus and washing with 1 per cent NaOH until filtrate is colorless	Wet........	Brown to black	Brown to black	Medium dark yellowish brown	Dark yellowish brown
	Dry.......	Brown tinged with black	Brown tinged with black	Medium dark yellowish brown	Medium dark yellowish brown

difference in the quantity of iron in the two varieties of tirs; in the gray tirs, however, the proportion of iron to alumina and colloidal silica is greater.

Because of their undesirable physical condition, the glei tirs are not suitable for agricultural purposes. Like all clay soils, they are very difficult to till. Being almost completely impervious and poorly drained, these soils are flooded during the rainy season. The surface swamping is accentuated by a high water table.

At least in the virgin state, the black tirs contain sufficient organic matter and hence nitrogen, for crop production. The supply of lime and iron is usually abundant in both the black and the gray tirs. The quantity of potash appears to be extraordinarily high in the hydrochloric acid extract, but nothing is known

[7] In tables 4 and 5 all the iron is expressed in Fe_2O_3. We have, however, always found considerable quantities of FeO in the glei tirs.

of its availability. The phosphoric acid supply seems to be insufficient, th figures varying between 0.21 and 0.03 per cent. Since the gray tirs has a lowe sesquioxides content, the Schneidewind quotient is more favorable in this so than in the black tirs.[8]

The chief advantage of the glei tirs for crop production is its high water table Under a xerophytic climate, the greatest yields are obtained in the driest years On the dess, which lacks such a water supply, the reverse is true. Yields o wheat on the black tirs vary from 10 to 25 m. q.[9] per hectare, though a recor crop of 32 m.q. has been reported on a farm east of the merdja Zaitrat on a soi corresponding to profile Gh-96. On the gray tirs the yields are less variable between 18 and 25 m.q. But the "good years" occur only two or three years ou of every ten.

The good crop years seemed to have encouraged the French pioneers to develop agriculture extensively in a country suitable for cattle and horse breeding. T< this purpose flood control has been instituted, and several drainage canals and many systems of ditches have been and are still being constructed.

On many tracts, such as those near the low Sebou and between Sidi-Slimar and Petitjean, irrigation works have also been installed, even on the black tirs where orange and other fruit trees have been planted. The occasional heavy rainfall, the impervious condition of the clay soil, and the rising of the ground waters in these areas have caused trouble. An increase of rising salts (chiefly NaCl) at the rhizosphere has been found to cause serious injury to the trees.

What has been said about the glei tirs of Morocco can be applied to all similar soils, which are widespread throughout the world, and of which the regur of India is a good example. Their several characteristics, black color, heavy texture, and tendency to crack, and the occurrence of salts have been difficult to explain. The clue, however, to their features and development is the role of the hydro-hypogenic activity in the soil-forming process.

BROKEN-LAND AND DEEP TIRS

The high and broken land which stretches east of the broad low plains of the tirs is cut by large rivers and valleys. In the bottomland, dess and the gray and other varieties of tirs may be found.

On the broken land the soils are, for the most part, dark to very dark in color. This region rises from less than 100 m. above sea level at the west to a maximum of 683 m. near Moulay-bou-Chta, south of Ouergha River. Geologically, the region is a heterogeneous mosaic of Eocene, Miocene (Burdigalian molasse, Vindobonian clays, marls, and conglomerates) and intra-Cretaceous formations, besprinkled by Jurassic and Triassic outcrops and even by eruptive patches. The natural vegetation is represented by forests of the *Olea-Lentiscus-Chamaerops* association. At present only remnants of these are scattered between the farm-steads.

[8] For the surface horizons of profiles Gh-107 and Gh-18 (tables 4 and 5) this quotient (which is considered as unfavorable from 60 upward) is respectively 89.7 and 81.2.

[9] Metric quintals, or 100 kgm.

Notwithstanding the heterogeneous character of the landscape, the profiles of most soils have one or more black or dark horizons. It is the calcareous nature of the material that is responsible for the accumulation of humus in these soils. Besides the dark color, these soils are endowed with a heavy clay texture, with frequent columnar structure, and with other concomittant features encountered in the black glei tirs. In many developed profiles, dry glei horizons are present, some with lime accumulation. It thus seems that, in this country, both the epipedic and the hypopedic processes have been acting together, the former tending to develop calcareous profiles, and the latter developing glei formations at some depth and bringing about a typical tirs upper horizon. Since these processes have not been constant with reference to time and place, the resulting profiles differ widely. Where the soils are very shallow, resting on the parent rock, which outcrops here and there, the black soils formed may be identified as rendzina. In other places, instead of the native rock, an unconsolidated mass of rounded pebbles occurs. By these, one may trace the ancient coastlines and the extent of the sea retreat to the west. A great part of the red sandy soil at the northern and eastern limits also has been formed on this coastline material. It is within this area that the sandy material, in turn, originated.

The following profile types of the broken-land tirs have been recorded:

I: black horizon; II: unconsolidated material
I: black horizon; II: brown transition horizon; III: unconsolidated
I: black horizon; II: brown transition horizon; III: dried mottled glei; IV: uncon-
solidated
I: black horizon; II: yellowish and gray glei, rather thick; III: not determined
I: black horizon; II: thin layers of unconsolidated material; III: white calcareous
accumulation layer; IV: C horizon of calcareous native rock (Eocene material in
the observed profiles, but having the appearance of a consolidated glei, with stony
consistency).

Where erosion has been active in these profiles, various underlying horizons, such as the transitional brown horizon, the consolidated glei, the calcareous accumulation, or the unconsolidated mass, have become exposed. Because of the outcropping of the unconsolidated materials, resulting from erosion caused by tillage and pasturing, the soils on the slopes have a stony appearance.

At many points on the bottoms of the broken land, a double calcareous crust has also been found under the black upper horizons of the glei tirs type. The upper layer of the crust is thin and very much indurated; the lower, much thicker and fairly soft, is formed only by the surface black clay horizon and has a more or less thick soft crust, a tuff-like formation. These are cases of mixed types of glei tirs and of crust tirs, which are discussed presently. Such crusts are, however, also found under the black mud sands.

Some profiles of this region are red below the upper black horizons. This coloration is due in some cases to the nature of the calcareous parent rock, the weathering products of which are red (lithochromic profiles). In other cases, chiefly adjoining the flat tirs, there are red sand profiles, of which the surface horizons have undergone or are undergoing tirsification.

All of these modifications indicate that the hydro-hypogenic process can influence the pedogenesis.

The broken-land tirs are less productive than those of the low plain, but are relatively not so poor for a xerophytic climate. On the shallow soils the crops are usually small, and even fail completely in unfavorable years. But where the black upper horizons are fairly thick, the yield of wheat, even under native culture, may be as high as 8 to 12, and in exceptional cases 15, m.q. per hectare.

At the bottoms of valleys and on low slopes in many places in this region, the black clay soil-forming process gives depth to the soils. Here the soils are much deeper than the black tirs of the low flat plains. Soils of the same type are also found in isolated tracts on level or on slightly undulating land, generally in slight depressions, as between Meknes and Fes. They are found more extensively on the flat plateau of Mershoush, west of Marchand, and on the piedmont plain of Kasba Tadla, mainly about Ghorm-el-Alem. These soils, known as "deep tirs," are by no means pocket formations. Their pedogenesis can be explained rather by the stability of the ground depressions and by the topsoil tirsification. On this soil the previously mentioned savanna of tall (2 to 3 m.) grasses and herbs, mainly umbellated and thorny composites, is encountered.

The black and clayey soil material thus formed may attain a thickness of several meters. In profile Mn-12, within the area of broken-land tirs, in the valley of the Wadi Rdom, 1 km. south of the Sidi Embarek station, 210 m. above sea level, the deep tirs horizons, formed on a substratum of unconsolidated materials, attain a thickness of 15 m., according to the owners. One can never be sure, however, of a layman's estimate of such matters. In several cases, for example in the deep tirs about Ghorm-el-Alem (profiles Ta-39 and Ta-40), a thickness of 2 to 3 m. has been ascertained. Below, lies a whitish crust about 1 m. thick, and farther down, an indurated mottled glei.

The depth of the water table varies markedly. In profile Mn-11, near Ain-Taomar, 25 to 26 km. southeast of Sidi-Sliman, 1 km. east of the road to Meknes at 390 m. above sea level, the water table on June 13, 1942, was at a maximum of 4.50 m. below the surface. The topography of this region is undulating, and in the lower levels of the same farm, the water table was at a depth of only 1.35 m. in June and came to the surface in winter. On the other hand, under profiles Mn-12, Ta-39, and Ta-40 no ground water table is known.

The texture of the black horizons of the deep tirs is on the order of that recorded for those of the low flat plain. Cracking, in the form of columnar macrostructure, appears from the surface downward in many profiles, such as Ta-39 and Ta-40, but not in others, as Mn-12, which usually remains fairly moist despite the lack of a known water table and the absence of irrigation. In Mn-11, though the water level was not very deep, the columnar structure was hardly discernible.

These soils contain more humus than any of the other tirs samples. In Mn-11, the humus content of the surface horizon (20 cm. deep) was 3.45 per cent, and the total organic matter content, 5.52 per cent; corresponding figures for horizon II (21-70 cm. deep) were 1.49 and 2.38 per cent, and for horizon III (71—more than 1 m. deep) 0.99 and 1.59 per cent. In Mn-12 the humus contents of four

horizons to a depth of 2 m. were respectively 1.96, 1.12, 0.96, and 0.78 per cent, and the contents of total organic matter, 3.14, 1.78, 1.54, and 1.25.

Many of these profiles, such as Ta-39 and Ta-40, contain no calcium carbonate. When present, it is similar to that in the tirs of the plains. The distribution of lime originates mainly from the hydro-hypopedic activity rather than from a leaching process. This is true also of soluble salts, which never occur in the upper levels in quantities injurious to plants.

The productiveness of the deep tirs is very high. On the farm from which profiles Ta-39 and Ta-40 were taken, the average wheat yield is 25 m.q. per hectare. The yield was 30 m.q. in 1939 when the profiles were studied, and the minimum yields were 6 or 7 m.q. in the most unfavorable years. This farm is not irrigated, and no manure is applied to the soil. On profile Mn-12 excellent crops of fruit (apricots in 1942) are also obtained under the system of dry farming. The rainfall in these two regions is only about 400 to 500 mm. and is very irregular, the summers being dry and hot. A moisture supply from below is the only possible explanation for the results.

CRUST TIRS AND MIXED SOILS

The crust tirs is a very different soil type from the glei tirs. The most typical of the crust tirs, designated as black, extend in a long band, parallel to the seacoast and behind the coastal strip of red sandy soils of the downs. Southeast of Casablanca this band attains a width of about 42.5 km.; and southeast of Mazagan, 85 km. From Safi southward, to its southern limit near Agadir, the band becomes progressively narrower, in places being broken into isolated tracts.

North of the crust tirs area, and between it and the glei tirs at the north, there is more or less broken land consisting of siallitic soils and soils formed from ancient rocks as substratum, interrupted by patches of red sandy soils and of glei and crust tirs.

The relatively uniform crust tirs formation is underlain by a nonhomogeneous substratum, which geologically is Quaternary or alluvial, partly Pliocene and Cretaceous. At its widest extent, in the north, the country is level or undulating, with 300 to 400 mm. of rainfall, in the domain of the *Olea-Lentiscus-Chamaerops* association. The southern tracts, forming level or undulating enclaves within a relatively broken landscape, have a rainfall of only 200 to 300 mm. and are in the domain of the *Argania spinosa* association, partly with the cactiform *Euphorbia Beaumierana*, and in many tracts intermixed with that of *Callitris articulata*. But most of these tirs areas, especially in the northern and widest part, were deforested long ago and are now cultivated mostly for cereal grains. Maize is also grown under a system of dry farming, and many tracts are in pasture.

A profile of typical crust tirs shows the following horizons:

One or two (rarely more) black or almost black upper horizons, usually tinged with chestnut or brown.

One or more crust horizons to be looked upon as calcareous hardpans. Usually, two types of crust are present. The upper crust is thinner and more indurated, of a stony consistency, and of a laminated or platy structure. The lower crust horizons, of which

there may be one or more, are commonly less indurated, of gritty microstructure, and of so great thickness that the pedological profiles, in places, cannot develop properly.

One or more glei horizons. As a rule, these form the lowest layers of any complete profile of crust tirs, since the crusts are but the result of glei formations which have indurated while the ground water level dropped to greater and greater depths. The glei horizons have been noted in 70 per cent of the profiles studied.

Analyses of profiles Cas-7 and Cas-8, typical examples of this crust tirs, are presented in tables 9 and 10. The crust and glei formations show that, in the pedogenesis of crust tirs, activity of deep-seated origin plays an important role. The upper parts of the profile, the black or very dark horizons, are rather epigenic, however, and differ strikingly from those of the tirs formations on the low flat plains.

The upper horizons of the crust tirs, unlike those of the glei, are not of a heavy clay texture. The structure is not of the columnar type. The humus content is always greater then 1 per cent—between 1.26 and 2.05 per cent—and generally

TABLE 9

*Mechanical composition of the upper horizons of crust tirs**

| | ATTERBERG'S FRACTIONS, PERCENTAGE OF THE TOTAL MECHANICAL FRACTION | | | |
	I (<2μ)	II (2–20μ)	III (20–200μ)	IV (200μ–2 mm.)
Sandy type: average of three horizons..............................	24.20	21.38	41.99	12.43
Clayey type: average of five horizons..............................	43.77	12.69	31.69	11.85
Average minimum....................	22.27	6.16	26.74	1.14
Average maximum..................	54.69	28.17	44.27	24.48

* Compare with figures in table 2.

is above the 2 per cent mark, with more than 0.1 per cent nitrogen. The carbonates ($CaCO_3$) vary widely, even in the edaphic (black) horizons: from less than 1 to 26.64 per cent; their distribution through each profile being also irregular. Thus, a leaching process might or might not have been effective, according to the predominance of the epigenic or hypogenic activity. In most cases the edaphic part of the profile is too thin to show the effects of the leaching process. In the crust, naturally, the carbonate may increase abruptly to more than 40 per cent. Soluble sulfates are generally absent or found in very small quantities. In all the black or A horizons analyzed, chlorides have been found, generally in quantities of less than 0.02 per cent (as NaCl), except in the surface horizon of profile Mog-1, where the chloride content was found to be 0.036 per cent. In horizons of lime accumulation, in crusts or glei, the amounts of NaCl vary from 0.006 to 0.690 per cent. In 62.5 per cent of the profiles analyzed the amounts are greater than 0.03, and in 12.5 per cent, greater than 0.1 per cent. The amounts of K_2O are relatively high, but those of P_2O_5 are low. The pH values vary between 7.2 and 7.45.

The striking difference, besides that of texture, between the black horizons of the crust tirs and those of the glei tirs is to be found in the hydrochloric acid

TABLE 10

Analysis of two black crust tirs profiles

Percentages of the 2-mm. sample dried at 105°C.

CONSTITUENTS	CAS-7*		
	I (0–28 cm.) A horizon	II (29–58 cm.) Crust	III (59–118 + cm.) Crust
Hygroscopic water†..................per cent	4.39	0.85	0.85
pH...	7.3	7.25	7.25
Total organic matter................per cent	2.45	....	....
Humus...........................per cent	2.05	....	....
Carbonates as CaCO₃...............per cent	1.04	40.34	41.88
Sulfates...........................per cent	0	0	0
Chlorides as NaCl..................per cent	0.018	0.068	0.032

	CAS-8‡			
	I (0–20 cm.) A₁ horizon	II (21–38 cm.) A₂ horizon	III (39–70 cm.) Crust	IV below (70 cm.) Crust
Hygroscopic water†..................per cent	5.37	5.21	2.99	2.62
pH...	7.4	7.45	7.3	7.25
Total organic matter................per cent	2.11	1.52	....	....
Humus...........................per cent	1.82	1.26	....	....
Carbonates as CaCO₃...............per cent	10.27	13.41	27.81	25.91
Sulfates...........................per cent	0	0	0	0
Chlorides as NaCl..................per cent	0.018	0.006	0.006	0.006

* Sample taken June 21, 1939, along the road to Marrakech, 34.3 km. from Casablanca and 7 km. before Ber-Reshid; 200 m. above sea level. Undulating plain. Alluvial substratum. Rainfall between 300 and 400 mm. In cereal grains with occasional association of *Chamaerops humilis*. Horizon I: chestnut-black; intermediate texture; subpolyhedral-fine structure (the smallest polyhedral lumps breaking down into dust); with abundant small gravel (1 mm.–1 cm.) containing calcareous concretions and small shell residues. Horizon II, stony crust, laminated in upper part. Horizon III, softer tufaceous crust.

† Determined May 17, 1940.

‡ Sample taken June 21, 1929, along the road to Mediouna, 16 km. north of Settat, under the same geographical conditions as those of Cas-7. In maize (dry farming). I, A₁ horizon: black tinged with violet-brown, mottled with yellow; intermediate texture; distinct platy structure, clods and lumps breaking into variously shaped figures of soft consistence; with abundant roseate-white, inconsistent calcareous nodule-shaped concretions. II, A₂ horizon, with a B-like appearance: medium-brown tinged with violet; more clayey; with subpolyhedral structure; calcareous concretions of several sizes, strongly consistent; and shell residues. Horizon III, soft crust: whitish, brownish, and roseate tinged; containing fine roots. Horizon IV, second soft crust: whitish, ocherous tinged; very strongly cemented by calcareous material; with distinct platy structure; and small lime-petrified plant residues.

extract, as may be inferred by comparing the figures of table 11 with those of tables 4, 5, and 6. In the typical profile Cas-7, very little, about 23 per cent,

of the fine fraction remains. In the glei tirs it varies from 45 to 64 per cent. As for the A horizons of the crust tirs, the content of the fine fraction approaches that of the fossil B and C horizons of the red sandy soils of the downs (10.4–13.2 and 20.2–33.4 per cent, respectively). The total cations, in milliequivalents per 100 gm., are also significant: almost 600, in comparison with more than 1100 in the glei tirs (300-404 for the B, and 431-751 for the C fossil horizons of the red sandy soils). The crust tirs appears to be sialferoid, since its sialferic complex is 75 per cent of the fine fraction. This amount is of the same order as those found in the A horizons of the glei tirs and in the B horizons of the red

TABLE 11

Chemical analysis* of the hydrochloric acid extract of horizon A of the black crust tirs profile Cas-7

PERCENTAGE COMPOSITION BY WEIGHT OF THE 2-MM. SAMPLE DRIED AT 105°C.		CATIONS	
Na_2O	0.40	Total.................... m.e./100 gm.	584.87
K_2O	0.52	Percentage of the cations:	
MgO	0.66	Na^+	2.19
CaO	2.91	K^+	1.89
MnO	0.76	Mg^{++}	5.63
Fe_2O_3	3.26	Ca^{++}	17.75
Al_2O_3	4.77	Mn^{++}	3.65
SO_3	0.11		
P_2O_5	0.04		
CO_2	1.20	Total monovalent and divalent	31.11
SiO_2	9.22		
TiO_2	n.d.		
Insoluble residue	70.98	Fe^{+++}	20.49
Loss on ignition	6.08	Al^{+++}	49.73
Total	100.91	Total trivalent	70.22
Fine fraction	22.94	Ratio monovalent and divalent/ trivalent	0.44
Sialferic complex, % of fine fraction	75.37		

* International method. For SiO_2/R_2O_3 see table 14.

sands. The ratio of monovalent and divalent to trivalent cations, 0.44, resembles more closely that of the glei tirs.

The SiO_2/R_2O_3 ratio of 1.41 in the crust tirs (see table 14), within the sialferic complex, distinguishes this tirs from the glei tirs, having a ratio varying from 2.13 to 3.23. In the SiO_2/Al_2O_3 ratio, the figure for the crust tirs, 3.28, is of the same order as that of the glei tirs, between 3.04 and 4.41. This is true also for the Al_2O_3/Fe_2O_3 ratio, 2.29 for the crust tirs and 1.72 to 2.22 for the gray glei tirs.

Intermediate profiles between those of the black crust and of the glei tirs are found in the northeastern part of the crust tirs area at the boundary of, or as an enclave within, the great siallitic central region, near Boulhaut. In these soils the double crust corresponds to the type of tirs crust. The black upper horizons

correspond to the glei tirs type, being very clayey and tending to crack into a columnar structure. The glei horizons are found usually at a relatively shallow depth. Thus these profiles should be grouped with the glei tirs as hydro-hypogenic soils. The characteristics of these tirs seem to bear a certain relation to the siliceous condition of the siallitic region and its neighboring areas: their black horizons are calcareous, in but few instances, but are more nearly siallitic, with CO_2 in quantities of less than 0.5 per cent, and their pH values seldom fall to 7.0. Their glei horizons, so far as is known, are sandy and of bluish tinge or mottled.

With the genuine black crust tirs must be grouped the chestnut tirs. Here and there, the two types are intermingled, the chestnut brown being found locally within the main area of the black tirs and with no definite boundaries. The chestnut tirs form a kind of transitional band lying between the black crust tirs and the soils toward the inland, fringing the southern boundary of the siallitic Oulmès Plateau, and covering the main stretch of the Tadla piedmont.

The chestnut crust tirs appear to form under the geographic conditions of a flat or undulating topography less than 400 to more than 800 m. above sea level, and with a rainfall of less than 200 to more than 400 mm. The substrata are partly of Quaternary formations, mostly unconsolidated materials, partly Upper Cretaceous or of the so-called "phosphate layers" (*couches à phosphates*, from Upper Cretaceous to nummulitic). The natural vegetation domain is that of the *Olea-Lentiscus-Chamaerops*, of *Callitris articulata*, and of *Zizyphus lotus* and *Pistacia atlantica* associations. In many tracts these domains have been interfered with, as may be observed even today in the scanty residues of vegetation left by general deforestation and tillage. At present the general landscape shows mostly cereal crops and grass and herb associations under sheep pasture. Remains of *Chamaerops humilis* are common in the west, and of *Zizyphus lotus*, in the east; and very rarely an *Oleaster*, *Callitris*, or *Pistacia atlantica* may be seen in the deforested area.

At the surface, the color of the chestnut tirs is always more or less dark, in some places as dark as that of the so-called black tirs, but variously tinged with black, pure chestnut, red, and brown. The A_2 horizons are often somewhat lighter. The crust horizons show two different types: one, similar to that of the black crust tirs; the other, nodular in character, the induration of the calcareous mass not forming simultaneously, but beginning with a formation of loose hardened nodules. The level of the ground water varies widely: in profile Ta-26 it stood at 7 m. on May 26, 1939, and at 2.50 after the parcel was irrigated; whereas under other profiles it lies below the 20 m. mark.

The texture of the A horizons is similar to that of the black crust tirs. Within this general class, the following types have been found in the A horizons analyzed: clayey sandy in 12.5 per cent; clayey silty fine sand in 25 per cent; sandy clayey in 25 per cent; fine-sandy clayey in 25 per cent; and fine-sandy-silty clayey in 12.5 per cent. Thus the texture is rather sandy in 37.5 per cent of the A horizons, and rather clayey in 62.5 per cent. Table 9 shows the mechanical composition of the two groups.

In the profiles studied, the humus content was 1.20 to 1.81 per cent. The organic matter content was therefore greater than 1.50 per cent in all profiles and exceeded 2 per cent in most. The percentages of nitrogen shown by the analyses varied from 0.079 to 0.147 in the A_1 horizons, but exceeded, in 83 per cent of the cases, the limit of 0.1 per cent conventionally set as necessary for crop production.

TABLE 12

Analysis* of the chestnut crust tirs profile Ta-28†

Percentages of the 2-mm. sample dried at 105-C.

		I (0–39 CM.) A₁	II (39–67 CM.) A₂(B)	III (67–94 CM.) CRUST	IV (94–115 + CM.) C
Organic matter (humus+)		1.46	1.01	0.14	0.23
Soluble materials		0.11	0.07	0.05	0.09
Carbonates as CaCO₃		32.00	17.80	33.60	23.70
Mechanical fraction		66.43	81.12	66.21	75.98
Atterberg's fractions, % of the total mechanical fraction	I (<2μ)	39.19	39.80	30.76	28.38
	II (2–20μ)	9.72	22.83	20.56	
	III (20–200μ)	37.30	31.25	37.17	56.25
	IV (200μ–2 mm.)	13.79	6.12	11.51	15.37
pH		7.45	7.5	7.48	7.30
Sulfates: water extract		0	tr?	tr?	0
Chlorides as NaCl		0.039	0.009	0.009	0.018
N		0.079	0.066	0.007	0.011
(N × 100)/Humus		5.4	6.5	5.0	4.8
Available K₂O		0.069	0.056	0.056	0.042
Available P₂O₅		0.071	0.040	0.032	0.016

* Results of analyses by the Centre de Recherches Agronomiques, of Rabat. Only the humus fraction of the organic matter was determined.

† Samples taken at Beni-Amir, in the vicinity of the high Oum-er-Rbia, in section VIII of irrigation area, 450 m. above sea level. Rainfall less than 300 mm. In maize. Profile sampled, May 26, 1939, before installation of irrigation works. Horizon I, dark reddish chestnut; sandy-clayey; prismatic-lumpy structure, readily breaking down into granules and fine particles. Horizon II, medium reddish brown; intermediate texture, cemented with lime; cloddy-platy-granular structure; present A_2 horizon, formerly B, tending to become a nodular crust. Horizon III, nodular crust in more advanced stage of development; medium brownish red; here and there small spots of weathered shells. Horizon IV, brown-red (typical shade of the "terra-rossa"); fine-sandy; polyhedral-cloddy structure, breaking down into granules and attaining a sandy loam texture; forming variously shaped lime concretions: remains of the red alluvium from which the soil seems to have formed. The actual glei lies very much deeper here.

Nearly all the profiles of this type are strongly calciferous; in 11.1 per cent of those examined, however, the CaCO₃ content was found to be moderate. In some, the CaCO₃ in the profile increases with depth. This, however, is not the general rule. As in the black crust tirs, the lime distribution is irregular, varying with each profile and among the profiles.

Analytical data on profile Ta-28 are given in table 12.

Under Mediterranean climatic conditions, the tendency is to form an epigenic calcareous profile, that is, one in which lime accumulation increases with depth. But in the chestnut tirs, the hydro-hypogenic process counteracts to some degree the effects of the epigenic. This fact is strikingly illustrated by the HCl-extract analyses of profile Ta-28 (table 13). The CaO content diminishes with depth, but the CO_2 varies without relation to the CaO. In comparison with molecular ratios, the proportions of CaO to $CaCO_3$ found in horizons I and II are higher, whereas those of CO_2 to $CaCO_3$ in III and IV are higher. The carbonate dis-

TABLE 13

Chemical analysis* of the hydrochloric acid extract of the chestnut crust tirs profile Ta-28†

PERCENTAGE COMPOSITION BY WEIGHT OF THE 2-MM. SAMPLE DRIED AT 105°C.					CATIONS				
	I (0–39 cm.) A₁	II (39–67 cm.) A₂(B)	III (67–94 cm.) Crust	IV (94–115 + cm.) C		I (0–39 cm.) A₁	II (39–67 cm.) A₂(B)	III (67–94 cm.) Crust	IV (94–115 + cm.) C
Na₂O	0.46	0.15	0.09	0.21	Total......... m.e./100 gm.	1,059.60	971.25	637.30	434.52
K₂O	1.46	1.14	0.82	0.97					
MgO	0.08	0.09	0.13	0.09	Percentage of the cations:				
CaO	23.34	16.51	13.13	8.42	Na⁺	1.40	0.50	0.46	0.87
MnO	0.03	0.05	0.03	0.01	K⁺	2.925	2.49	2.73	4.74
Fe₂O₃	1.75	3.09	1.60	1.23	Mg⁺⁺	0.375	0.46	1.01	1.03
Al₂O₃	1.88	3.93	1.38	1.00	Ca⁺⁺	78.57	60.63	73.49	69.12
SO₃	0	0	0	0	Mn⁺⁺	0.08	0.15	0.13	0.06
P₂O₅	0.11	0.18	0.13	0.02	Total monovalent and divalent	83.35	64.23	77.82	75.82
CO₂	14.10	7.80	14.80	10.40	Fe⁺⁺⁺	6.21	11.96	9.44	10.64
SiO₂	2.77	1.80	1.52	0.92	Al⁺⁺⁺	10.44	23.81	12.74	13.54
TiO₂	n.d.	n.d.	n.d.	n.d.					
Insoluble residue	47.01	55.88	44.68	74.08	Total trivalent	16.65	35.77	22.18	24.18
Loss on ignition	6.97	9.37	12.21?	2.59					
Total	99.96	99.99	90.52?	99.94	Ratio monovalent and divalent/trivalent	5.01	1.71	3.51	3.14
Fine fraction	46.02	34.75	43.11	23.33					
Sialferic complex, % of fine fraction	14.15	25.90	10.74	13.59					

* International method. For SiO_2/R_2O_3 see table 14. Percentages by weight determined by the Centre de Recherches Agronomiques, of Rabat. The total for horizon III indicates an error in analysis or in transcription. If the error is in the insoluble residue, which probably ought to be 54.68 instead of 44.68, the fine fraction becomes 33.11, and the sialferic complex, 14 per cent of the last amount.

† For profile description, see table 12.

tribution, therefore, does not appear to be due to epigenic leaching from the surface downward. Rather, the CO_2 is brought upward from below in the form of bicarbonate, and it is in the upper horizons that it changes to the stable carbonate and later accumulates. The CaO/CO_2 ratio of $CaCO_3$ is 1.274 on a molecular weight basis, whereas it is 1.655 in horizon I, 2.117 in II, 0.887 in III, and 0.810 in IV. The increase of CaO, from I to II, might perhaps be ascribed to epipedic leaching; but in this case, the partial accumulation with increasing depth in the upper layers of the profile concerns only the CaO, not the $CaCO_3$ since CO_2 diminishes. In the black tirs profile Cas-7 (see table 11) an excess of

lime in the upper (edaphic) level of the profile (horizon A) is found. There the CaO/CO_2 ratio is 2.425. Thus, there is no question that in the black and the chestnut tirs an epigenic and a hypogenic process are active, the intensity of the processes varying with locality and time.

As in the black tirs, the quantity of sulfate in the chestnut tirs is negligible. The chloride content is also of the same order as that in the black tirs. In the 63 horizons of chestnut crust tirs studied, no traces of chloride were found in 23.8 per cent, less than 0.03 per cent (measured in NaCl) in 92 per cent, and from 0.03 to 0.3 per cent in 8 per cent. None of the soils had a chloride content higher than 0.3 per cent. In 23 profiles in which the so-called available potash was determined by the official laboratories at Rabat and Casablanca, the amounts varied from 0.012 to 0.105 per cent, exceeding in 87 per cent of the cases the limit of 0.04 per cent conventionally set as necessary for crop production. In six determinations, made at Rabat, of available P_2O_5 in surface horizons, the amounts varied from 0.021 to 0.071 per cent, the conventional limit of 0.07 per cent being exceeded in only one profile. In 16 A horizons, analyzed at Casablanca by the strong acid extraction method, the amounts were from 0.067 to 0.497, the limit of 0.25 per cent being exceeded in 55.5 per cent of the profiles.

The pH values of the upper horizons in these soils are of the same order as those in the black crust tirs, varying from 7.2 to 7.65, being equal to or greater than 7.3 in 92.6 per cent of the cases.

A comparison of the HCl-extract analyses of the standard black tirs profile Cas-7 (table 11) and the chestnut crust tirs profile Ta-28 (table 13) seems to show striking differences. In Ta-28-I the fine fraction is much greater than in Cas-7-I, the percentage of the sialferic complex (related to the fine fraction) is much smaller, the sum of the monovalent and divalent cations is much greater, and that of the trivalent much smaller, and the ratio of monovalent and divalent to trivalent cations more than 10 times higher. These apparent differences are due only to the amount of carbonates, and chiefly of CaO, which as a rule are very irregular in these soil types and therefore have no typological value. When this factor is eliminated, the incompatible figures of tables 11 and 13 change to those of table 14, becoming of almost the same category or, at least, thoroughly compatible within a particular typological unit. The main difference is apparent in the amount of mobile SiO_2, which is rather high in the black crust tirs. This characteristic brings the soil nearer to the glei tirs type. The silica-sesquioxide ratio is rather low in Ta-28-II, which seems to be a fossil B horizon, that is, one of sesquioxide accumulation, formed in a more humid climatic period. In Ta-28-I, the figures appear to be nearly the same as in Cas-7-I.

As has been shown, the upper horizons, black or chestnut, of the crust tirs may be more or less influenced by hydro-hypogenic activities, but their soil-forming process is mainly epigenic. On the "Hauts Plateaux" of North Africa, crusts, indurated at a certain depth, often outcrop as a result of erosion. These outcroppings are later covered by sands, from which evolve the red soils. It is possible that, in the crust tirs areas, the same phenomenon occurs in places, chiefly in the eroded tirs area. But these are only relics of erosion in the

general landscape. The upper horizons of crust tirs in the black or chestnut types originate mainly under forest. Examples attesting to this fact are innumerable from the northeastern extremity, in the *Olea-Lentiscus-Chamaerops* and *Callitris* domains, to the outermost southwestern limits of the area, under *Argania* forest. Near the northeastern limit, beginning about 17.1 km. northeast of Tadla, the Taquibalt forest still persists. On the plant map of Emberger, it falls within the domain of the *callitretum*. All through the area, starting at the ancient rock plateau 4.7 km. beyond the northeastern limit, the author noted

TABLE 14

Comparison of certain ionic relations and molecular ratios of typical black and chestnut crust tirs

		CAS-7	Ta-28			
		I—A	I—A₁	II—A₂(B)	III—Crust	IV—C
Percentages of HCl extract, on carbonate-free basis						
Fine fraction after removal of carbonates or bicarbonates corresponding to amount of CO_2........		20.211	12.957	17.013	....	4.174
Percentage of foregoing total	Fe_2O_3....	16.13	13.50	18.16	....	31.22
	Al_2O_3....	23.60	14.51	23.16	....	23.98
	SiO_2.....	45.62	21.37	10.58	....	22.06
	Total....	481.07	227.07	382.34	....	134.18
M.e. of cations without Ca^{++}	Fe^{+++}, %	25.47	28.96	60.49	....	34.45
	Al^{+++}, %.	58.35	48.72	34.75	....	43.86
Ratio of monovalent and divalent/trivalent ions after equalization of the Ca of Ta-28 and Cas-7......		0.45	1.35	0.60	....	1.01
Molecular ratios						
SiO_2/R_2O_3..........................		1.41	1.57	0.52	1.08	0.88
SiO_2/Al_2O_3..........................		3.28	2.50	0.78	1.87	1.57
SiO_2/Fe_2O_3..........................		7.52	4.21	1.55	2.53	1.99
Al_2O_3/Fe_2O_3.......................		2.29	1.69	1.99	1.35	1.27

the association of *Olea-Lentiscus-Chamaerops*, accompanied by other woody species such as *Ceratonia siliqua*, *Rhamnus oleoides*, and *Phillyrea angustifolia*. Under this cover, now open and degenerated into arbuscular form, the soil, in general, is similar to that of Ta-33, a typical profile, 22 to 23 km. northeast of Tadla, and 625 to 650 m. above sea level:

I—30 to 50 cm. thick. Dark brown with a violet tinge; of an intermediate texture; crumby to granular structure; abundant plant residues.

II—25 to 30 cm. thick. Indurated, stony crust, mottled chiefly with pink and white, showing in the natural position an arrangement of parallel plates.

III—Beginning at a maximum depth of 80 cm. and extending downward. Softer crust with a tuff-like microstructure; irregular consistency.

The glei was not found here. The whole profile lacked sulfates and showed but very small quantities of chlorides. The pH values of the three horizons were 7.55, 7.55, and 7.52.

This typical profile is definitely of the crust tirs type. Like may other samples, it shows that crusts can be formed under forest. It might be added that this forest region is spotted throughout by "daias," small depressions (dry at the time of the author's visit) of glei-like origin, the surface of which breaks into polygons. The hydro-hypopedic process acts here despite the general substratum of Upper Cretaceous limestone.

At the other extremity of the area, between Safi and Agadir, several analogous profiles were examined. These had black or dark brown upper horizons, double or triple crust and glei formations, under arganietum, *Zizyphus lotus*, and other tree covers.

The dark color of the crust tirs, black or chestnut, is usually more or less of a reddish shade. These soils do not belong to the cyanic series, whereas the glei tirs do. The crust tirs have been affected to some degree also by hydro-hypogenic interference. Wherever this activity failed to reach the surface, the explanation of the dark color is similar to that given for the upper horizons of many pedocals.

In places, the crust tirs merge, without sharply defined boundaries, into other soil types, such as glei tirs or pedocals. An especially good example of such mergence is found in the plain at the foot of Tadla, where a soil mosaic is formed on the substratum of Quaternary alluvia. This mosaic shows chestnut tirs; spots of deep glei tirs; black or very dark crust profiles, the upper horizons of which are partly of intermediate texture, partly clayey, partly merging to solon-chak with efflorescenses at the surface; and finally, red calcareous soils, some with crusts and some of the "terra-rossa" type, merging into chestnut tirs.

Within the great arc formed by the chestnut tirs band, toward the southwest, extends a large inland region of soils that are for the most part degraded and eroded. In the northeastern part, largely north and east of El Borouj, these soils are underlain principally with Upper Cretaceous rocks and phosphate layers; in places Quaternary unconsolidated materials may be encountered. The topography of the region is rolling, and the height above sea level is from 500 to 800 m. The annual rainfall varies from less than 300 to more than 400 mm., and summers are hot and dry. The original plant cover, now almost extinct, consists of *Olea-Lentiscus-Chamaerops* and *Callitris* climax in the northern tract and of *Zizyphus lotus* and *Pistacia atlantica* steppe-forest elsewhere. The soils are calcareous, mostly shallow, with outcropping crusts, brought about by erosion, and intermixed with gravel and pebbles. Almost all of the area is devoted to cereal grains and to sheep grazing.

The color of the A horizons is medium to nearly dark brown, and in places is tinged with black or red. In general, the color is lighter than that of the tirs previously discussed. This is to be expected, since the humus content of these soils has been reduced. The texture of this horizon is generally midway between light and heavy. The crusts are similar to those found among the younger tirs:

a stone-like thin platy crust, overlying a tufaceous and much thicker crust. Glei is always found at the bottom wherever the crust formation is deep enough. The carbonate content of the A horizon varies; there is virtually no sulfate; the chloride content is small, the maximum being 0.02 per cent. All of these characteristics agree with the essential features of the chestnut crust tirs described.

Among the profiles studied in this area, OZ-1 is of special interest. It was located 1.25 km. northwest of Oued-Zem, 800 m above sea level, on an area of

TABLE 15

Analysis of the degraded crust tirs profile OZ-1

Percentages of the 2-mm. sample (for the A horizons) dried at 105°C.

	A HORIZONS		CALCAREOUS DEPOSIT
	I (0–25 cm.)	II (25–45 cm.)	III (45–105 cm.)
Hygroscopic water (January 9, 1941)	1.34	2.08	2.79
pH	7.3	7.3	....
Carbonates as $CaCO_3$	7.316	20.723	58.29
Soluble sulfates	0	0	....
Chlorides as NaCl	0.018	0.006	....

	CRUSTS		
	IV (1.05–2.05 m.)	V (2.05–2.51 m.)	VI (2.51–3.31 m.)
Hygroscopic water (January 8, 1941)	1.70	1.15	2.18
Carbonates as $CaCO_3$	61.91	56.39	32.178

	GLEI		
	VII (3.31–3.96 m.)	VIII (3.96–3.98 m.)	IX (3.98–4.28 + m.)
Hygroscopic water (January 8, 1941)	1.99	10.47	4.17
Carbonates as $CaCO_3$	32.416	18.406	51.692

Upper Cretaceous limestone, with 400 mm. of rainfall and a poor vegetation cover of herbs. The profile showed the following horizons:

I—20 to 25 cm. thick, A_1 horizon. Medium brown, of an intermediate texture and crumb-granular structure.

II—20 cm. thick, A_2. Rather dark brown, with prismatic laminar structure.

III—60 to 65 cm. thick, calcareous deposit. Tufaceous, with concretional microstructure and laminar cleavage.

IV—100 cm. thick indurated crust. Brown, rather sandy, with calcareous cement, and laminated or platy structure.

V—40 cm. thick, more massive crust. Brown with pink spots, coarse sand with calcareous cement, fissured on the exposed surface, tending to prismatic structure.

VI—80 cm. thick, crust somewhat similar to horizon V. Roseate, with white horizontal streaks.

VII—65 cm. thick, glei horizon. Greenish brown, rust-spotted, the exposed surface exfoliating in scale-like fashion.

VIII—2 cm. thick. Two streaks, whitish above and dark below, separating the two glei horizons.

IX—30 cm. thick, young glei. Moist, rusty, with gritty microstructure.

Analyses supplementing the description of this profile are given in table 15.

This profile shows clearly the last phases of the hydro-hypogenic process, becoming regressive. The ground water level has fallen, leaving behind glei formations, which subsequently have indurated successively in the form of crusts. The carbonate contents of the three upper horizons might be considered as the result of an epigenic process of leaching and accumulation. From horizon IV downward, a different picture is presented. The increase from III to IV is insignificant compared to that between I and III. From horizons IV to VIII the amount decreases from 61.9 to 18.4 per cent; at horizon IX the percentage again increases abruptly to 51.7. Each glei horizon, and therefore each crust, appear thus to be independent of the others. The composition of each one depends on the nature and force of the hypogenic process at the time of each formation.

As to the fertility characteristics of the normal crust tirs, the improved physical condition, in comparison with that of the glei tirs, has been pointed out. The nitrogen content is usually higher than 0.1 per cent wherever the surface horizons have not been disturbed. The potash content is generally above the requirement conventionally set as the limit for crop production. The P_2O_5 content, however, is generally below the limit, except where phosphate layers occur. The soil productiveness is also influenced by other conditions. The total thickness of the black or chestnut horizons varies from a few centimeters to more than 1 m., and in places where the crust outcrops, these horizons are lacking completely. The depth of the ground water varies widely, being generally deep, with interposition of thick crusts. The climatic characteristics, such as the rainfall, also show variations. Perhaps more than anything else, the degree and the nature of the erosion complicate the picture. The cropping characteristics, therefore, differ strikingly throughout the whole extent of the crust tirs. In all tracts of the black tirs area in the northwest and in several isolated deep and moist patches in the plains at the foot of Tadla and Beni-Mellal, European farming flourishes. Irrigation has been successfully practiced in both the European and the native farm areas. On the other hand, in many inland tracts on the same plain at the foot of Tadla, wheat yields average 7 to 9 m. q. for European farming and 5 to 6 for native cultivation. The large differences depend mainly on the rainfall. In most areas of the eroded tirs, the natives receive poor returns for wheat, and good ones for barley.

THE ACTIVITY OF MICROORGANISMS IN THE TRANSFORMATION OF PLANT MATERIALS IN SOIL UNDER VARIOUS CONDITIONS[1]

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The fact is well established that numerous agents influence the activities of soil microorganisms engaged in the decomposition processes. Less well understood, however, is the variability of the influence of these agents when any one is altered. The varied nature of these influences has been the subject of numerous reports, the majority of which have dealt with the chemical aspects of decomposition. Of those concerned with the microorganisms engaged in decomposition, only a few have given detailed information. Early investigations dealt with the influence of soil amendments on microbial activity but failed to note microbial changes during the early stages of decomposition. More recent reports, however, have yielded information concerning these earlier stages. Smith and Humfeld (8, 9) noted that during the decomposition of green manures, the numbers of bacteria followed carbon dioxide production, which rose rapidly during the first 4 days then declined sharply to a fairly constant level. Vandecaveye and co-workers (12, 13, 14, 15), who used mature plant material as a source of organic matter, failed to note this correlation but observed that the maximum number of microorganisms occurred several days after carbon dioxide production had declined. The explanation for such differences may lie in the fact that wide differences existed in the soils involved, in the type and physical conditions of the plant materials used, and even in the methods employed. It is of interest to note, however, that Allen *et al.* (1), working under conditions varying greatly from those of the aforementioned investigators, found a positive correlation between numbers of bacteria, losses in organic matter, and carbon dioxide production. Furthermore, Smith and Brown (7) and Corbet (2) reported that carbon dioxide evolution from a soil resembled the type of carbon dioxide curve observed in pure cultures of bacteria. It would seem, therefore, that increased carbon dioxide production would be paralleled by an increase in numbers of microorganisms.

The experiments reported herein show that if dry, finely ground plant material is added to a relatively small volume of soil in the laboratory, the results are comparable to those from experiments wherein green, succulent material is added to soil in greenhouse benches (8).

PLAN OF EXPERIMENT

One-thousand-gram samples of screened and thoroughly mixed Leonardtown silt loam from the University farm, were placed in 5-pound glass chemical bottles and treated with plant materials and inorganic fertilizers. The bottles were divided into two series, one receiving ground corn stover and the other ground mature red clover, each being applied at the rate of 1 per cent by weight. Plant materials were ground in a Wiley mill to pass through a 10-mesh screen. The following treatments were made in duplicate:

1. Control—no treatment.
2. Corn stover.
3. Corn stover + ammonium nitrate.
4. Corn stover + ammonium nitrate + lime.
5. Corn stover + ammonium nitrate + lime + monocalcium phosphate.

The same treatments were repeated with red clover in place of corn stover. Ammonium nitrate was applied to supply nitrogen at the rate of 3 per cent by weight of the plant materials added, or 0.87 gm. per kilogram of soil. Lime in the form of calcium oxide was added at the rate of 2 gm. per kilogram, and monocalcium phosphate was added at the rate of 1.8 gm. per kilogram of soil. Enough water was added to bring the moisture content of the soil up to two thirds of the water-holding capacity and maintained at that moisture content by periodical additions of water.

Samples for microbiological studies and moisture determinations were taken at the beginning and after 3, 6, 12, 24, 54, 84, and 144 days of incubation. During sampling the soils were removed from the jars, thoroughly mixed, sampled, and replaced. Duplicate plate counts were made for total number of bacteria, actinomyces, and fungi using soil extract agar, sodium asparaginate glycerol agar, and potato dextrose agar, respectively. Soil extract agar and sodium asparaginate glycerol agar were adjusted to pH 7.2–7.4. Potato dextrose agar was acidified to pH 4.3–4.5 by the addition of sterile lactic acid just before the plates were poured. All plates were incubated at 30°C. The numbers of bacterial colonies were counted after an incubation period of 7 days, fungi after 3 days, and actinomyces after 9 to 10 days. Counts on the actinomyces were made by the aid of a low-power microscope. Moisture determinations were made on the soils at the time of plating, and the numbers of microorganisms were calculated in terms of oven-dry soil.

By means of a modification of Heck's (7) method, carbon dioxide determinations were made on the soil during the first 17 days. Air drawn through the bottles was not previously purified, but controls were run and the necessary corrections made for atmospheric carbon dioxide. Titrations were made daily for the first 9 days, after which they were made at 48-hour intervals.

From time to time, determinations of the pH of the soil were made with a Beckman pH meter and glass electrode, a soil:water ratio of 3:2 being used. All studies were carried on simultaneously for 17 days, after which carbon dioxide determinations were discontinued.

RESULTS

Influence of various soil treatments on bacterial activity

Quantitative determinations of the bacterial content of soils under various treatments are shown in figure 1. Numbers of bacteria in these soils rose rapidly during the first 3 days of the experiment. Thereafter, the numbers dropped rapidly to the sixth day and declined gradually during the remainder of the
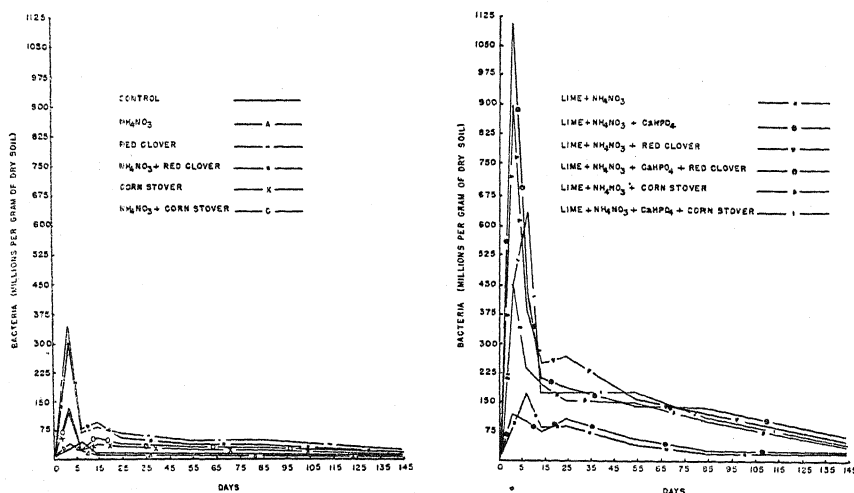
FIG. 1. NUMBERS OF BACTERIA IN LEONARDTOWN SILT LOAM TREATED WITH CORN STOVER OR RED CLOVER AND VARIOUS MINERAL FERTILIZERS

experiment. This rise and fall occurred regardless of the type of treatment, although the extent of the rise varied greatly.

The addition of plant materials to the soil produced a rapid increase in bacterial numbers, the greatest increase being produced by red clover. Numerous early reports have indicated that mature leguminous plants stimulate activity to a greater extent than mature nonlegumes (5). The increased stimulation by legumes appears to be due to differences in chemical composition of the two materials rather than to differences in nitrogen content (4). The fact that added nitrogen in the form of ammonium nitrate failed further to stimulate bacterial activity lends support to this view.

When lime was added to the soil containing decomposable material, bacterial activity was greatly stimulated. Red clover plus lime produced the greatest stimulation, maintaining about the same relationship with corn stover plus lime as existed between the two materials without lime. Lime alone caused

some increase in numbers of bacteria.   This observation was made by early soil microbiologists, who attributed it to changes in soil reaction which favored bacterial development.   Humfeld and Smith (3) and Thom and Smith (10), however, have found that the soil reaction has only a slight influence on microorganisms active during the early stages of the decomposition of plant materials. They observed that only those microorganisms on or in the immediate vicinity of the plant substance were stimulated.   Only when the plant materials are thoroughly and intimately incorporated with the soil will the soil reaction have a more pronounced effect.   As indicated in figure 1, the addition of ground mature red clover to the soil caused a much greater increase in bacterial numbers than did ground corn stover.   The addition of lime caused a great increase in numbers of bacteria, regardless of the type of plant material present.   However, there were roughly twice as many bacteria in the soil containing red clover as in the soil containing corn stover, either in the presence or in the absence of
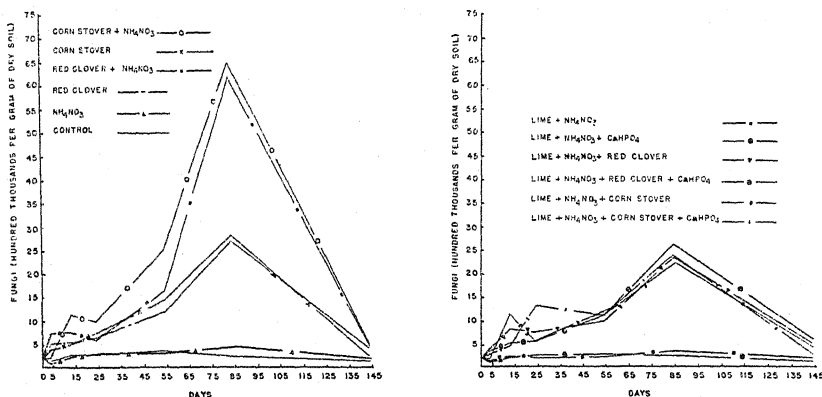
FIG. 2. NUMBERS OF FUNGI IN LEONARDTOWN SILT LOAM TREATED WITH CORN STOVER OR RED CLOVER AND VARIOUS MINERAL FERTILIZERS

lime.   The addition of monocalcium phosphate caused a slight increase in the total numbers of bacteria, the greatest occurring in the presence of red clover. It appears, from these results, that the type of plant material as well as the addition of lime plays an important role in influencing the numbers of bacteria in this soil.

Influence of the various treatments on numbers of fungi

The results in figure 2 show that the numbers of fungi did not increase appreciably during the early stages of decomposition.   The rise was gradual to 54 days, after which the increase was more rapid until a maximum was reached in 84 days.   Inorganic fertilizers appeared to have a greater influence on the fungi than did the type of plant material.   There appeared only slight differences between the two series, but the addition of ammonium nitrate had a marked stimulatory effect on the numbers of fungi.   This stimulation was expected for two reasons: first, fungi require more nitrogen than do bacteria, since the former

produce more cellular material; and second, the addition of ammonium nitrate would increase soil acidity and, therefore, be less favorable to bacterial development. The addition of lime, however, completely nullified the beneficial in-

TABLE 1

Numbers of actinomyces in soils treated with various plant and inorganic materials

Counts, on duplicate bottles, in millions per gram of oven-dry soil

TREATMENT	NUMBERS OF ACTINOMYCES							
	0 days	3 days	6 days	12 days	24 days	54 days	84 days	144 days
No treatment	.64	1.057	1.540	3.21	1.32	1.87	4.69	1.27
	.64	2.370	1.280	2.72	2.85	0.94	2.82	1.80
Red clover	.64	0.270	0.380	1.81	2.40	2.19	5.00	1.41
	.64	. . . . .	. . . . .	1.67	4.17	2.09	6.50	2.15
Corn stover	.64	. . . . .	. . . . .	0.64	1.47	3.41	1.35	2.38
	.64	0.810	0.96	0.91	1.66	1.14	3.87	1.07
Ammonium nitrate	.64	2.140	1.03	4.32	2.15	1.58	3.37	0.47
	.64	0.948	0.51	2.02	1.95	1.24	3.62	1.31
Red clover + ammonium nitrate	.64	0.135	0.64	1.95	2.72	1.16	2.06	0.81
	.64	. . . . .	0.57	1.19	2.22	. . . .	. . . .	0.27
Corn stover + ammonium nitrate	.64	0.545	0.51	1.74	3.54	0.77	3.00	0.67
	.64	0.272	1.48	1.95	1.39	1.21	2.56	0.40
Ammonium nitrate + lime	.64	0.945	0.77	1.88	1.81	0.92	2.56	2.27
	.64	1.080	1.67	2.02	2.64	1.66	3.62	1.61
Red clover + ammonium nitrate + lime	.64	0.270	3.28	3.41	2.75	3.69	25.25	13.06
	.64	0.540	0.57	2.31	1.85	3.11	28.69	6.72
Corn stover + ammonium nitrate + lime	.64	0.472	0.75	2.37	4.03	4.81	19.62	13.13
	.64	0.135	0.64	4.81	2.85	3.68	24.56	10.72
Ammonium nitrate + lime + phosphate	.64	0.475	1.60	2.30	2.50	1.03	3.12	2.35
	.64	0.675	1.22	2.37	2.43	1.75	4.06	0.97
Red clover + ammonium nitrate + lime + phosphate	.64	. . . . .	1.86	2.65	3.89	4.94	36.78	19.29
	.64	0.270	0.71	3.14	1.75	6.37	33.38	18.70
Corn stover + ammonium nitrate + lime + phosphate	.64	. . . . .	0.64	3.00	3.75	5.63	32.51	18.16
	.64	0.880	0.64	8.02	4.55	7.44	34.25	9.78

fluence of ammonium nitrate. Apparently this reaction was not due directly to the difference in pH, because at 54 days the pH of the limed soil was about the same as that of the unlimed soil. The initial shift in pH, however, may have enabled bacteria to use most of the available food material. The addition of

monocalcium phosphate produced a slight, though probably insignificant increase in numbers of fungi. Smith and Humfeld (9) found little change in numbers of fungi. Vandecaveye and Villanueva (12) reported a maximum number of fungi in field soils 60 to 70 days after manure was applied. Later, in laboratory experiments Vandecaveye and Allen (13) and Vandecaveye and Katznelson (15) found the maximum numbers of fungi to occur in 20 to 40 days.

Influence of the various treatments on numbers of actinomyces

The results of plate counts of actinomyces (table 1) indicated only a slight stimulation, especially in the early stages of decomposition. In the limed soils, the numbers of actinomyces showed a definite increase after 54 days, reaching a maximum in 84 days. There was little difference between the two plant mate-
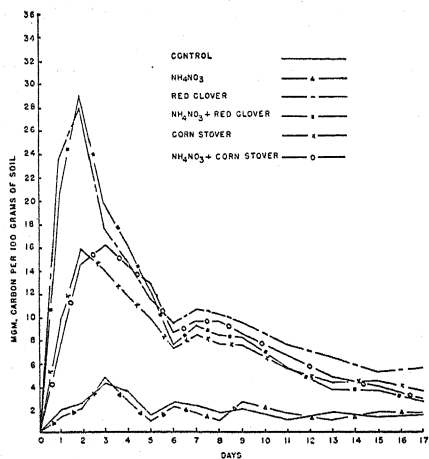
FIG. 3. MILLIGRAMS OF CARBON AS CARBON DIOXIDE EVOLVED FROM LEONARDTOWN SILT
LOAM TREATED WITH CORN STOVER OR RED CLOVER AND AMMONIUM NITRATE

rials, although the numbers in the clover series were slightly higher than those in the corn stover series. The results indicate that of the inorganic materials added, lime exerted the greatest influence.

Influence of the various treatments on carbon dioxide production and on pH

The results of carbon dioxide measurements are shown in figures 3 and 4. The rapid rise in carbon dioxide production and the almost equally rapid fall during the first few days of decomposition have been noted by Smith and Humfeld (9), Vandecaveye and co-workers (12, 13, 14, 15), and many others. This type of curve is indicative of the "explosive" nature of decomposition processes during the early stages when the more soluble materials are present. A comparison of figure 1 with figures 3 and 4 shows that the production of carbon dioxide was closely paralleled by numbers of bacteria. It is of interest to note that after the fourth day carbon dioxide evolution was greatest in the corn stover series. The numbers of bacteria during the corresponding period were about the

same in both series. Similar results on carbon dioxide production have been noted by Turk and Millar (11) and Millar, Smith, and Brown (6). These investigators attributed the reduced carbon dioxide evolution in legume decomposition to a greater fixation of carbon in the soils.
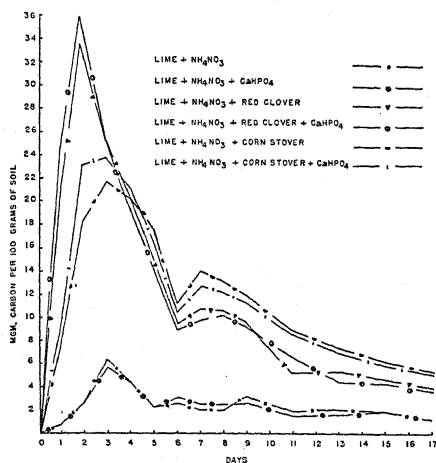
FIG. 4. MILLIGRAMS OF CARBON AS CARBON DIOXIDE EVOLVED FROM LEONARDTOWN SILT LOAM TREATED WITH CORN STOVER OR RED CLOVER, LIME, AMMONIUM NITRATE, AND MONOCALCIUM PHOSPHATE

TABLE 2

pH values of soils treated with variou plant and fertilizers materials

Average of duplicate samples

TREATMENT	pH			
	0 days	3 days	54 days	144 days
No treatment	5.2	5.16	4.91	4.84
Red clover	5.2	5.58	5.32	4.94
Corn stover	5.2	5.32	5.40	5.12
Ammonium nitrate	5.0	4.93	4.35	4.45
Red clover + ammonium nitrate	5.0	5.33	4.40	4.46
Corn stover + ammonium nitrate	5.0	4.90	4.50	4.45
Lime + ammonium nitrate	7.25	7.35	5.10	4.92
Red clover + ammonium nitrate + lime	7.25	7.16	5.42	5.25
Corn stover + ammonium nitrate + lime	7.25	7.12	5.60	5.40
Ammonium nitrate + lime + phosphate	7.10	7.30	5.00	4.85
Red clover + ammonium nitrate + lime + phosphate	7.10	6.91	5.25	5.19
Corn stover + ammonium nitrate + lime + phosphate	7.10	6.75	5.44	5.33

The pH values of the variously treated soils at 0, 3, 54, and 144 days are shown in table 2. These results indicated that liming raised the pH only temporarily. Smith and Humfeld (9), however, noted little or no change in pH in the soil they used. This difference may be explained by the fact that they used Leonardtown

clay loam, which has a greater inorganic colloid content than the Leonardtown silt loam used in the experiments reported herein. Furthermore, ammonium nitrate was added to the limed soils, which when completely converted to the nitrate form would have a tendency to make the soil acid.

The effect of lime on fungi and actinomyces apparently can not be attributed directly to differences in pH of the soil. The effect may be explained, however, by the fact that the addition of lime increased the activity of bacteria, which utilized the food needed for the growth of fungi. In the competition for food between groups of microorganisms a change in fertilizer and liming practices may favor one group or the other. Such practices will undoubtedly play an important role in the conservation of soil organic matter.

SUMMARY

Small amounts of soil treated with various inorganic fertilizers and with dry, finely ground plant materials were subjected to a microbiological study in an attempt to determine the type of microflora active at various stages of decomposition, and to determine the effect of two plant materials of widely differing chemical composition with and without various fertilizers on the soil microflora.

The results showed that the numbers of bacteria rose to a maximum by the end of 3 days. Thereafter, the numbers dropped rapidly to the sixth day and declined gradually during the remainder of the experiment. After 54 days the numbers of fungi and actinomyces began to increase and reached a maximum at the 84th day.

The numbers of bacteria were affected most by the nature of the plant material and by the application of lime. The addition of lime produced slightly greater increases in the red clover series than in the corn stover series. The type of plant material, however, had little influence on the numbers of fungi and actinomyces. Ammonium nitrate produced the most favorable influence on fungi, but its influence was completely offset by the addition of lime. Lime, however, produced an increase in the numbers of actinomyces.

Studies showed that the evolution of carbon dioxide was closely correlated with fluctuations in numbers of bacteria.

The lime produced only a temporary change in pH values of the soil. The addition of lime appears to have had a retarding influence on fungal development, as a result of increased bacterial activity rather than of differences in the pH level of the soil.

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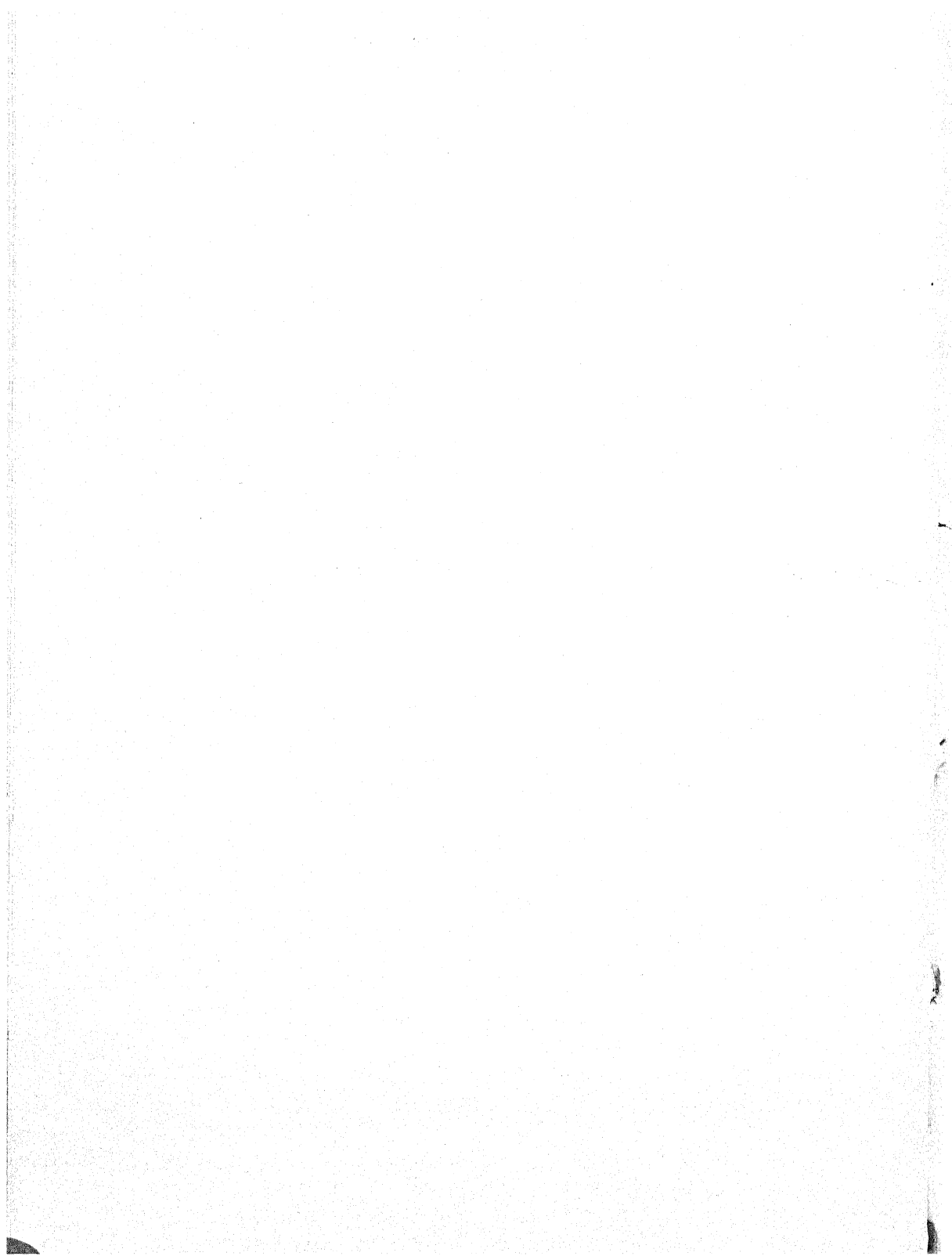
MICROBIAL ACTIVITY AND AGGREGATION OF MIXTURES OF BENTONITE AND SAND[1]

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The number of water-stable aggregates in soils has been found by various workers (3, 4, 5, 7) to be closely associated with microbial activity. The metabolic products of the organisms are believed to be responsible for the increased aggregation. The influence of the activity and the general types of organisms has not been determined quantitatively under controlled conditions. It is the purpose of this paper to report the relationships of the aggregation of sand, clay, and organic matter mixtures to microbial activity as determined under controlled laboratory experimentation.

EXPERIMENTAL METHODS

The basic mixture used in these experiments consisted of dune sand (15 gm.) and bentonite clay (5 gm.). The sand was screened to pass a sieve of 0.25-mm. mesh but retained by one of 0.15 mm. To this mixture 1.5 gm. of organic matter, corresponding to a rate of 8 tons per acre inch, was added. Organic matter increments consisted of finely ground alfalfa and corn stover, both untreated and hot-water-extracted. The water-extracted sample added was equivalent in weight to the untreated sample. After thorough mixing, the sample was moistened with 10 ml. of $(NH_4)_2HPO_4$ solution (0.071 gm.) and 10 ml. of a 1 to 10 dilution of a suspension of 50 gm. of Grundy silt loam to 580 ml. of water. Samples were incubated in a nearly saturated atmosphere at a temperature of 28°C. for 3,000 hours.

At intervals during the incubation, triplicate samples were removed and dried above a hot plate. The samples were passed through a 4-mm. sieve, duplicate 10-gm. samples were weighed out, and aggregate analysis was determined by wet sieving using a 0.25-mm. sieve (2). After 20 minutes of oscillation at the rate of thirty-eight $1\frac{1}{2}$-inch strokes per minute, the material remaining on the screens was dried and weighed. Replicates varied from 0 to 17 per cent of the mean value, with an average value of 2.5 per cent. The weight of aggregates remaining on the screens was corrected for the amount of material found on the screen if no incubation occurred, i.e., the initial sample was subjected to identical aggregate analysis. The average of the corrected weights is reported as the percentage of water-stable aggregates greater than 0.25 mm. attributable to biological action.

Carbon dioxide was determined during the period of incubation by collection and subsequent aeration of prepared triplicate samples in stoppered bottles (6). Standard NaOH was used as the CO_2 absorbent and was titrated with standard HCl, phenolphthalein being used as the indicator.

EXPERIMENTAL RESULTS AND DISCUSSION

The rate of carbon dioxide evolution and the percentage of aggregates greater than 0.25 mm. at the various intervals for the samples containing alfalfa and corn stover are shown in figures 1 and 2.
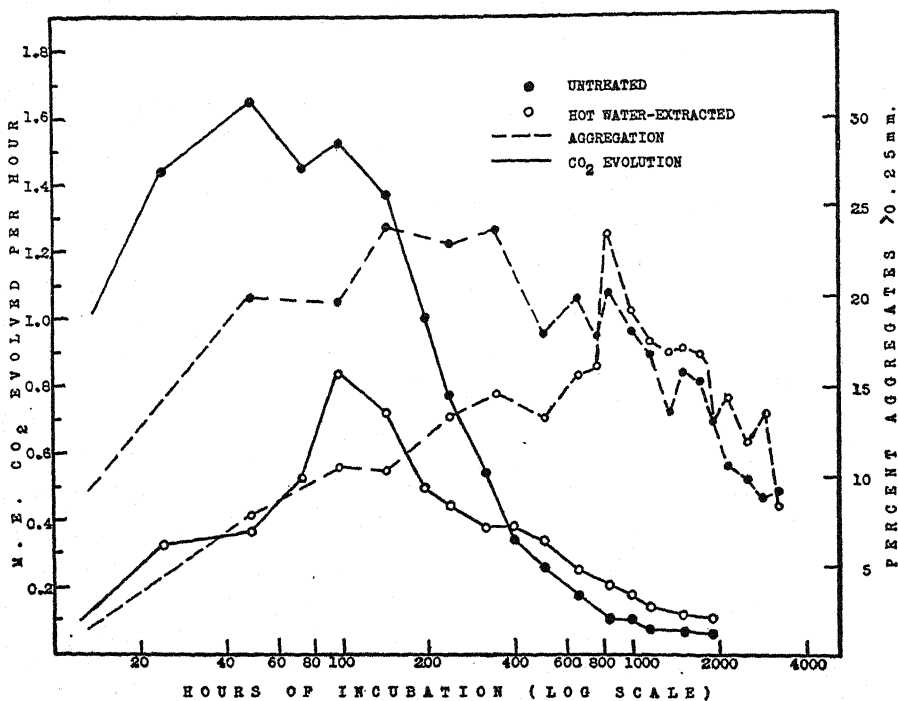
FIG. 1. RATE OF CARBON DIOXIDE EVOLUTION AND PERCENTAGE OF AGGREGATES GREATER THAN 0.25 MM. FOR PUDDLED MIXTURES OF BENTONITE CLAY AND SAND WITH ALFALFA

The curves for CO_2 evolution are considered typical of the material used (6). The difference in readily available material, as reflected by microbial activity, between the two types of organic matter is apparent as well as the changes due to the hot water treatment. The untreated samples provide more readily available food, causing the microorganisms to multiply rapidly and become active in a comparatively short time. In the water-extracted samples the increase of microbial activity, as measured by CO_2 evolution, is delayed, as less readily available food is present. As decomposition proceeds, however, additional material becomes available for bacterial use, and the microbial activity increases.

This study was chiefly concerned with the changes of aggregation during organic matter decomposition. Aggregate stability was found to increase with incubation time, to reach one or more maximums, and then to decrease. The water-extracted samples exhibit one prominent maximum at 816 hours; the untreated corn stover sample exhibits maximums at 144 and 816 hours, and the untreated sample of alfalfa, a maximum at 144 hours and a plateau in the 816-hour zone. The position of these maximum aggregation percentages follows the peaks of CO_2 evolution in all cases by a definite time interval.
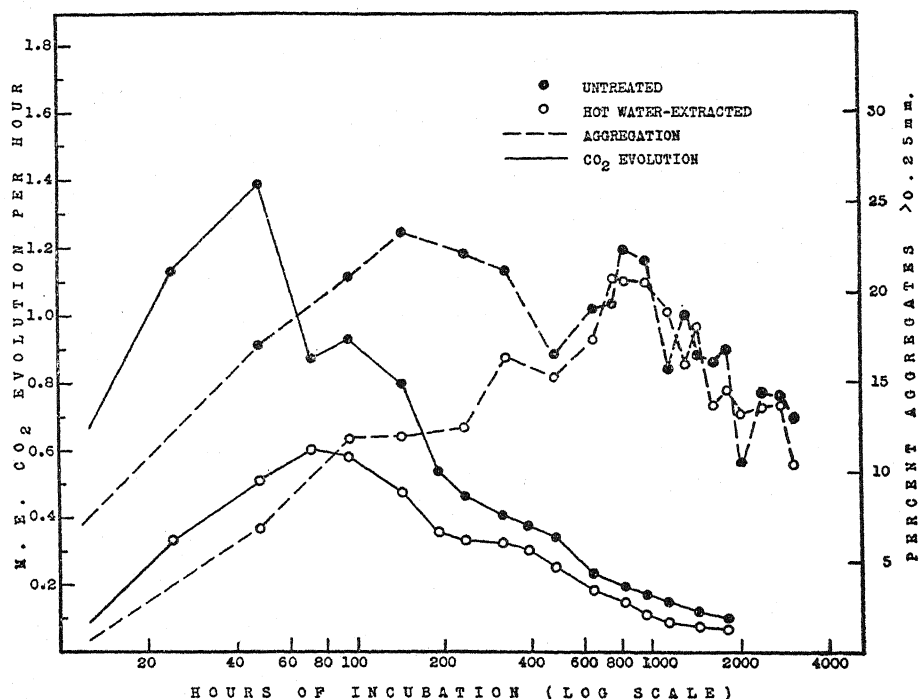
FIG. 2. RATE OF CARBON DIOXIDE EVOLUTION AND PERCENTAGE OF AGGREGATES GREATER THAN 0.25 MM. FOR PUDDLED MIXTURES OF BENTONITE CLAY AND SAND WITH CORN STOVER

The first peak of aggregation, shown only by the untreated samples, is due to the decomposition of the readily available plant materials. A huge bacterial population arises during this initial decomposition process. As the supply of readily available food is quickly used up, microbial activity drops. A considerable quantity of microbial wastes has accumulated, however, and this is now augmented by the multitudes of dead microbial tissues due to lack of sufficient energy materials to continue life. These materials, colloidal in nature, now permeate the soil mixture and impart a relatively stable aggregation for a time. This initial maximum of water-stability was found to occur in 6 days.

The increased aggregation attributed to the wastes resulting from decompo-

sition of the most readily available plant foods is of short duration. By 20 days the degree of water stability had greatly lessened. Myers and McCalla (5) report a maximum aggregation at 8 days for soils treated with peptone and/or sucrose. Martin and Waksman (4) report that in most of their experiments maximum aggregation occurred after 10 to 25 days' incubation. Browning and Milan (1) found maximum aggregation to develop within 20 days under the conditions studied. Peele and Beale (8) report increased aggregation of Cecil sandy loam during the entire growing season with the addition of sucrose. The last experiment, being conducted on a field scale, did not allow for strict control of conditions affecting microbial activity. Under field conditions so many variables are encountered that it is difficult to isolate the effects of the factors involved. It is difficult, therefore, to compare the results with those obtained in the various controlled laboratory experiments.

During the period of decline in the stability of the soil aggregates formed by the initial decomposition processes, microbial activity continues. Organic matter decomposition proceeds less spectacularly than during the initial period. One microbial population follows another as the composition of the organic material present is altered. Their numbers decrease, as does the activity expressed by CO_2 evolution. While the initial water-stability lessens, the decomposition of the less readily available fractions of the organic materials, as well as the decomposition products and microbial wastes and bodies from the more readily available portions, proceeds. Decomposition products and microbial metabolic wastes gradually accumulate and give rise to a slowly increasing aggregation, as shown by the increasing stability of aggregates in the water-treated samples. After 20 days, the increasing aggregation due to the decomposition of the less readily available organic matter fractions exceeds the diminishing aggregation due to the initial decomposition of the most readily available materials. The increase in aggregation of the water-extracted samples is gradual and does not have the initial stability shown by the untreated samples. The second maximum stability occurs after 34 days. In the case of the untreated alfalfa sample, no definite maximum occurs, but a plateau does appear just prior to this sampling date, and the stability of the samples decreases subsequently as with all the treated samples. As the aggregation of the water-extracted alfalfa sample shows a marked maximum at this point, it is thought that the plateau signifies an increased stability of the aggregates due to the accumulation of metabolic products and decomposition of the less readily available material. A previously conducted experiment with corn stover gave the same general results, two maximums of aggregate stability with the untreated samples and only the second one with the water-extracted samples.

Observations during the course of the experiment showed that after some 300 hours' incubation the growth of fungi was very marked. This growth is reflected in the CO_2 evolution curves, which show a leveling in the vicinity of 300 to 400 hours' incubation. One material, the untreated alfalfa sample, does not show this change in the CO_2 production curve. Since this same sample fails to show a definite second maximum of aggregate stability, it is thought that the decom-

position of the fungal bodies and their wastes may play an important role in causing the second aggregation maximum.

After reaching the peak at 816 hours the precentage of water-stable aggregates decreases steadily for the remainder of the incubation period. The soil population is still changing and is active, as shown by the continued CO_2 evolution. Although the rate of CO_2 evolution is low, it should be remembered that the time intervals have greatly increased and therefore the total CO_2 evolved is still a sizeable quantity. The decrease in aggregation after 34 days is less rapid than after the 6-day peak. As the activity of the microbial population declines, less rapid disintegration is to be expected. Furthermore, the types of material involved in this decomposition would be assumed to be less easily decomposed. It is believed that this decrease in water-stable aggregates is due, as in the case of the decrease of the initial peak aggregation with the untreated samples, to the gradual microbial decomposition of the various materials forming the cementing agents. As the populations change, each successive one tends to work on the wastes created by the previous one as well as on the portions undecomposed by the preceding populations. Hence destruction of the cementing agent in the soil aggregate causes a decrease in the water-stability of the aggregate.

Previous work (5) had indicated that aggregation was associated with bacteria only insofar as these organisms are responsible for the accumulation of certain metabolic products that function as cementing materials. Peele (7) showed that mucus produced by bacteria could be utilized as a binding agent with soil or quartz sand to provide water-stable aggregates. Also the data presented in this paper indicate that it is the decomposition of the bacterial tissues and wastes, superimposed on that of the organic matter, which produces maximum aggregation.

Soil aggregation, due to the organic matter decomposition by microorganisms, is always changing. New organic matter additions provide energy for new populations, which give rise to decomposition products and wastes, which are, in turn, a source of energy for another microbial population. Accumulations of these decomposition products, microbial wastes, and dead microbial tissues provide a colloidal cementing agent. Water-stable aggregates are developed as these materials are formed. The stability of these aggregates decreases as these materials are further decomposed.

The aggregation produced by the two types of organic matter decomposition processes described is not additive. Each cycle of decomposition gives rise to a maximum water-stability of its own, regardless of other processes. This is well illustrated by the data. The aggregation curves for the hot-water-extracted materials increase regularly to their peak, with no indication of any prior peak. On the other hand, the curves for the untreated samples show the initial peak, then decline despite the fact that it can be assumed the aggregation due to decomposition of the less readily available materials is increasing at the same time. Only when the two curves cross is it apparent in the untreated samples that aggregation has been increasing because of the decomposition of the less readily available fractions. Martin and Waksman (4) report that aggregation

produced by composted manure was greatest after 150 to 210 day's incubation, and that up to 150 days, added peat had no effect. Such slowly decomposed organic materials produce aggregation slowly, if at all. Browning and Milam (1) show that peat and other such less readily decomposed materials produce no appreciable increase in aggregate stability in a year's time.

GENERAL DISCUSSION

The results reported are those for ideal conditions for microbial activity. The organic matter was finely ground, the temperature was high and constant, and moisture was near optimum. Under these conditions the rate of decomposition would be expected to be most rapid. It may be expected, therefore, that in the field the time required for the various peaks of microbial activity and water-stability of the aggregates to occur will be longer and the peaks more irregular in sequence. Furthermore, because of these irregularities, the various highs may be difficult to distinguish. Under normal conditions, however, it would be expected that after an organic increment to the soil a rapid increase in aggregation would occur, particularly if the amount of easily available energy material for bacterial use was high and if conditions for microbial activity were favorable. This aggregation would be brief and would be followed by a decline and then a second increase in the percentage of water-stable aggregates. The second period of increased stability of the aggregates would be more lasting. After this a gradual decrease would result. These periods of maximum aggregation will not occur, it is assumed, at regular intervals, but will occur as conditions permit. Type and fineness of organic matter, temperature, and humidity are probably the limiting conditions. Materials high in nitrogenous tissues would be assumed to decompose more quickly than carbonaceous materials, with the result that the initial peak of water-stability would occur sooner and the magnitude would be greater.

SUMMARY AND CONCLUSIONS

The relationship between water-stable aggregation and microbial activity as measured by CO_2 evolution was studied for mixtures of bentonite clay, sand, and finely ground alfalfa and corn stover, untreated and hot-water-extracted.

It was found that during 3,000 hours of incubation two peaks of water-stability of aggregates occurred, both as a result of an accumulation of decomposition products and metabolic wastes due to microbial action.

In the presence of much readily decomposable organic material an initial peak of aggregation occurs under laboratory conditions in the space of 6 days. This aggregation is attributed to the decomposition products and microbial wastes from the rapid decomposition of the easily decomposed plant tissues. The aggregation disintegrates in a short time and is superseded by a second rise in aggregation. This peak of aggregation, the only one shown by the samples containing little of the easily decomposable material, occurs after 34 days. This maximum is attributed to the accumulation of decomposition products and microbial wastes from the slower decomposition of the less readily decomposed organic

materials. These aggregates disintegrate, in turn, although more slowly than with the first maximum.

The two maximums of aggregation are not additive. The factors producing the one apparently do not influence the other, nor are they influenced by the factors producing the other.

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THE ACTION OF CALCIUM CYANIDE AS A SOIL DISINFECTANT[1]

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That calcium cyanide has not been used extensively as a soil disinfectant is evinced by the limited number of reports on the subject in the literature. Although the toxic effect of the cyanide upon insects and microorganisms is well recognized (14), we are not aware of any study of the availability of the nitrogen in the cyanide when it is added to the soil or of the influence of this disinfectant upon nitrification in soil. These two points received attention in the experiment reported in this paper, following field tests (4) which showed better growth of pineapple plants in plots treated with Cyanogas[2] than in control plots. The availability of the nitrogen of thiocyanates in soil and their effect on nitrification have been elucidated by Sandhoff and Skinner (13).

Perotti (11) in 1920 made the observation that KCN in concentrations of about 0.05 per cent serves as a nitrogen nutriment for some types of microorganisms. Burnet (2) later differentiated bacteria into two distinct groups by their reaction to cyanide in solid media under aerobic conditions: (a) those relatively sensitive to inhibition by cyanide and (b) those insensitive to cyanide. Respiration studies in recent years (3) consider the cyanides to be inhibitory for those organisms containing cytochrome. The specific action of cyanide is the inactivation of indophenol oxidase (cytochrome oxidase), thus effectively inhibiting respiration through cytochrome.

Rao and Rao (12) furnished evidence that the introduction of cyanides into soil may affect the process of biological nitrification of ammonium nitrogen. It was shown that concentrations as low as $0.000025M$ cyanide inhibit the growth and respiration of nitrite-forming bacteria in flasks. The reduction in loss of nitrogen from 30.4 to 13.3 per cent in manure heaps with 0.5 per cent cyanide, as reported by Kudzin (9), may indicate a similar function of cyanide.

Cyanides in soil treatments have decreased populations of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey (10), the sugarbeet nematode, *Heterodera schachtii* Schmidt (5, 17), and wireworms (8). In limited experiments with the pineapple plant (4) definite superiority of plant growth and fruit yields in soil treated with calcium cyanide over untreated plants was observed.

METHODS

Preparation and treatment of soil

A lateritic soil of pH 4.4 from an upland pineapple-producing area was passed through a $\frac{1}{4}$-inch mesh screen, and 35 pounds of the soil (moisture 19.72 per cent)

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[1] Published with the approval of the acting director as Technical Paper No. 152 of the Pineapple Research Institute, University of Hawaii.

[2] A product of the American Cyanamid Company containing not less than 40 per cent and not more than 50 per cent of calcium cyanide, $Ca(CN)_2$.

was placed in each of 45 enamel pots. To 15 pots ammonium sulfate was added at the rate of 156 p.p.m. nitrogen on the dry soil basis. To another group of 15 pots Cyanogas was added at the rate of 80 p.p.m. nitrogen.[3] This included 72 p.p.m. nitrogen in the form of cyanide and 8 p.p.m. in the form of ammonium, since analyses indicated that the Cyanogas contained 6.33 per cent nitrogen as cyanide and 0.67 per cent nitrogen as ammonium. A third group of 15 pots remained as untreated, unfertilized checks.

Following addition of the ammonium sulfate and Cyanogas, all pots were thoroughly wetted with $2\frac{1}{2}$ liters of tap water. The pots were then covered with mulch paper of the asphalt-impregnated type commonly used in pineapple culture. The covers were removed 7 days after treatment was started. The pots were then watered weekly to their maximum water-holding capacity. Soil samples were taken at intervals for determination of pH, amounts of ammonium and nitrate, and microorganic populations.

Analytical methods

Soil ammonium nitrogen was determined by displacement with a 10 per cent solution of KCl and distillation after addition of MgO (7). Nitrate was extracted with distilled water and determined by the phenol-disulfonic acid method (6). Soil pH was determined with the glass electrode using a soil-water ratio of 1:1. All soil analyses were made on triplicate samples, each sample being composited from five individual pots.

Cyanide was determined by titration with $AgNO_3$ (1). Ammonium nitrogen in Cyanogas was determined by aeration at room temperature after the solution was made alkaline with an excess of MgO. Pure samples of NaCN run concurrently indicated no conversion of cyanide to ammonium nitrogen under the conditions of the analysis.

Numbers of bacteria, fungi, and actinomycetes were estimated by the agar plate method. Bacteria and actinomycetes were grown on a soil extract medium, and fungi were grown on an acid agar medium with dextrose and peptone as sources of nutrients. Recommendations for the use of sufficient replicates, range of dilutions, incubation periods, and limits of numbers per plate were observed in making the counts (18).

RESULTS

Nitrification of added ammonium sulfate

Table 1 shows that the check pots without added fertilizer remained continuously low in both soil ammonium and nitrate. In contrast, the pots that received ammonium sulfate showed large amounts of ammonium initially and these gradually decreased toward the end of the experiment. Concomitant with reductions in ammonium, increasing amounts of soil nitrate were detected in this

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[3] It had been intended to apply equivalent amounts of nitrogen as ammonium sulfate and as cyanide. Subsequent analysis of this particular lot of Cyanogas showed that about half of its nitrogen had been lost, presumably as a result of storage for a long time under prevailing warm, moist conditions.

treatment. Ammonium decreased from an initial value of 129 p.p.m. to 4 p.p.m., while nitrate increased from 30 p.p.m. to 102 p.p.m. at 26 weeks. Apparently, active nitrification and nearly complete disappearance of the ammonium took place in the 26-week period. This is fairly rapid in view of the low pH of 4.4, which is close to the minimum of pH 3.7 to 4.0, below which nitrification does not proceed (18).

Full recovery of the nitrogen added as ammonium sulfate was not obtained at any time. The highest recovery was at the 16-week sampling period when 88 per cent of the calculated amount of nitrogen added was recovered. A small part of the unrecovered nitrogen in the earlier samplings may have been tem-

TABLE 1

Available nitrogen (nitrate and ammonium) in soil after addition of nitrogen as ammonium sulfate and Cyanogas

WEEKS AFTER TREATMENT		4	8	12	16	20	26
Analysis	Treatment*	Nitrogen in soil, p.p.m.					
Nitrate	$(NH_4)_2SO_4$	30	42	72	117	103	102
	$Ca(CN)_2$	11	9	10	23	15	21
	Check	21	20	24	31	28	31
Ammonium	$(NH_4)_2SO_4$	129	121	83	67	33	4
	$Ca(CN)_2$	80	92	90	94	101	77
	Check	13	10	12	16	12	2
Total available nitrogen ($NO_3 + NH_4$)	$(NH_4)_2SO_4$	159	163	155	184	136	106
	$Ca(CN)_2$	91	101	100	117	116	98
	Check	34	30	36	47	40	33
Recovery of available N from $(NH_4)_2SO_4$ relative to amt. of N added†...per cent		80	85	76	88	62	47
Recovery of available N from $Ca(CN)_2$ relative to amt. of N added†......per cent		71	89	80	88	95	81

* $(NH_4)_2SO_4$, 156 p.p.m. N added; $Ca(CN)_2$, 80 p.p.m. N added; check, no nitrogen added.
† Recovery in each case calculated on basis of nitrogen found above that in check.

porarily in the form of nitrite, for which no analysis was made. The low recovery of total available nitrogen during the 20- and 26-week samplings was probably the result of slow but continued transfer of nitrate nitrogen from the top layers of soil to the bottoms of the containers by the weekly applications of water.

Recovery of nitrogen from cyanide

Between 71 per cent (at 4 weeks) and 95 per cent (at 20 weeks) of the nitrogen added to the soil in the form of Cyanogas (6.33 per cent N as CN^- and 0.67 per cent as NH_4^+) was recovered in the form of soil ammonium nitrogen. It is possible that some of the nitrogen recovered in this treatment may have come from the inactivation and subsequent decomposition of soil organisms that were

cyanide-sensitive. A concurrent experiment using the same soil, with chloropicrin as the disinfectant, indicated that the maximum amount of nitrogen from this source did not exceed 12 p.p.m. On this basis, the maximum amount of nitrogen recovered from cyanide would not be more than 80 per cent.

The experience of Sandhoff and Skinner (13) indicated that the recovery of nitrogen from sodium thiocyanate depended partly on the concentration used. Little of the theoretical thiocyanate nitrogen could be recovered as ammonia or nitrate when 1 per cent sodium thiocyanate was added to unlimed garden soil, but when only 0.075 per cent was added to limed soil, an average of 37.1 per cent of the theoretical thiocyanate radical was recovered as ammonium or nitrate.

The nitrogen in the Cyanogas treatment remained as ammonium, since the values for nitrate throughout the 26-week period did not exceed those for the check treatment. Cyanide apparently acted as a powerful inhibitor of the process of nitrification in this soil under the conditions prevailing. Whether the
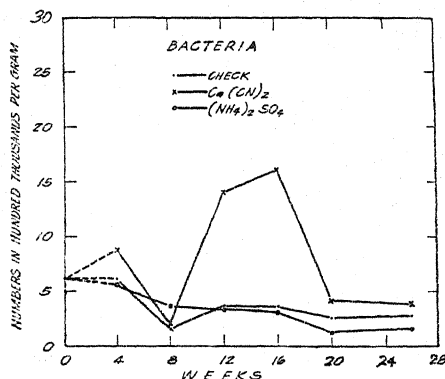
FIG. 1. NUMBERS OF BACTERIA IN UNTREATED SOIL AND IN SOIL TREATED WITH CALCIUM CYANIDE AND WITH AMMONIUM SULFATE

action was primarily on the nitrite-forming bacteria, as was shown by Rao and Rao (12) under artificial conditions, or whether it affected the nitrate-forming bacteria was not determined, since no analyses were made for nitrite in this experiment.

Effect of cyanide on numbers of soil microorganisms

The numbers of bacteria, fungi, and actinomycetes determined by the agar plate method over the 26-week period are presented in figures 1 to 3. Judging from the increase in numbers of bacteria following treatment, it appears that this was the group of microorganisms chiefly affected by cyanide treatment of the soil. Past experience with other disinfectants showed that stimulation usually followed an initial drastic reduction in numbers of microorganisms. Although a slight reduction is indicated at the 8-week sampling, it appears that the cyanide treatment was not primarily responsible for this action, since the count in the check treatment was also low. Although cyanide may not be so powerful a soil

disinfectant as, for example, steam and chloropicrin, it is selective in nature, as shown by the inhibition of the bacteria involved in the oxidation of soil ammonium nitrogen. This is not surprising, since only those organisms presumably depending chiefly on cytochrome oxidase for their energy would be affected by cyanide.

Cyanide as employed in the present experiment appeared to be nontoxic to soil fungi, on the basis of the total numbers represented in figure 2. Examination of the agar plates, however, indicated that some species were suppressed and that, as a result, the remaining unaffected species were favored. Although the over-all quantity was not altered, the number of representative genera was reduced.

There seemed to be a slight stimulation in the numbers of actinomycetes in the cyanide-treated soil (fig. 3). The increase in numbers between the 8- and 20-week sampling periods, however, was not large.
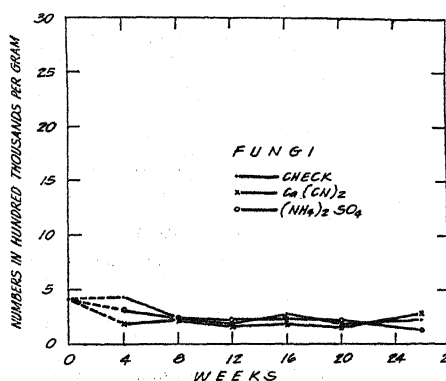
FIG. 2.   FIG. 3.

FIG. 2. NUMBERS OF FUNGI IN UNTREATED SOIL AND IN SOIL TREATED WITH CALCIUM CYANIDE AND WITH AMMONIUM SULFATE
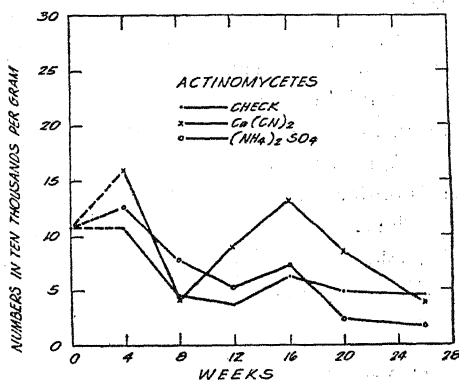
FIG. 3. NUMBERS OF ACTINOMYCETES IN UNTREATED SOIL AND IN SOIL TREATED WITH CALCIUM CYANIDE AND WITH AMMONIUM SULFATE

DISCUSSION

The conversion of a large part of the cyanide nitrogen of Cyanogas into ammonium nitrogen and the continued inhibition of nitrification for at least 26 weeks are of importance aside from any effect of the disinfectant on soil pathogens. The results suggest that if the pineapple, which absorbs and assimilates nitrogen more rapidly in the form of ammonium than of nitrate (15), had been grown in soil treated with cyanide, a more rapid and darker green growth would have resulted than if grown in nondisinfected soil. Such a response has been shown (16) with other soil disinfectants which inhibited nitrification. It is possible, also, that the greater growth and yield of pineapples previously observed (4) in the field after treatment with Cyanogas was in part related to the availability of some of the nitrogen from the cyanide.

Although the Cyanogas inhibited nitrification for a period, a similar effect was

noted (16) after disinfection with chloropicrin, steam, and formaldehyde as well as certain other compounds.[4] Cyanogas differed from all of these in that it contributed a considerable amount of nitrogen to the soil.

The growth of any plant in soil treated with the cyanide will undoubtedly be influenced by a number of factors, such as (a) the effect of the disinfectant upon any soil pathogens which might restrict the growth of the plant, (b) the response of the plant to ammonium as contrasted with nitrate nutrition, (c) the relationship of pH of the culture medium to ammonification and nitrification, and (d) the recovery of nitrogen from cyanide under conditions prevailing in the field.

SUMMARY

Calcium cyanide in the form of the commercial product, Cyanogas, was applied to a Hawaiian lateritic soil of pH 4.4 at the rate of 80 p.p.m. of nitrogen. Under the conditions of the experiment, with abundant moisture and a mulch-paper cover, 81 per cent of the nitrogen in the Cyanogas was recovered as ammonium in the soil 26 weeks after its application.

Complete elimination of the nitrifying organisms in the soil was indicated by failure to detect appreciable nitrate formation for 26 weeks after Cyanogas treatment, in contrast with nearly complete nitrification of added ammonium sulfate in nondisinfected soil.

The cyanide had relatively little effect on populations of fungi and actinomycetes. Only bacteria showed a limited increase in numbers after treatment with Cyanogas.

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DEVELOPMENT AND USE OF A POWDERY INDICATOR FOR RAPID AND ACCURATE ESTIMATION OF SOIL REACTION

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Various types of apparatus and reagents designed to meet the demand for a simple, rapid, and reasonably accurate method of measuring soil pH in the field are on the market. Among them, the Hellige and Truog soil reaction sets deserve special mention. Since these sets are offered for commercial purposes, the nature and composition of their reagents have not been made known.

During the summer of 1940, the senior author examined the soils of the experimental fields of the National Tea Experimental Station at Meitan. A test made by the Truog method showed that the plots in which tea seedlings grew poorly had pH values above 8.5, whereas those in which the tea seedlings grew vigorously had pH values below 6.5. These results confirmed the reports from tea soil experts that high acidity is one of the determining factors in the growth of the tea plant. A plan was then made to test in detail the soils of the entire experimental station, involving about one hundred acres of hilly land.

With a view to preparing a mixed indicator similar to the Truog reagent, so that tests of soils could be carried out on a large scale in a simple way, the present investigation was undertaken.

PREPARATION AND USE OF THE LIQUID INDICATOR

Trials of a number of indicators showed three, bromcresol green, bromcresol purple, and cresol red, to be satisfactory. One-tenth gram of each of these was ground together in an agate mortar with 5.9 cc. of 0.1 N NaOH and a few cubic centimeters of distilled water and the mixture diluted to 400 cc. Buffered solutions of pH 4.0, 4.4, 4.8, 5.2, 5.6, 6.0, 6.4, 6.8, 7.2, 7.6, 8.0, 8.4, 8.8, and 9.2 were prepared as prescribed by Clark and Lubs. When five drops of the indicator solution was added to 5 cc. of each of the buffered solutions in Pyrex test tubes, a series of colored standards varying from orange-brown to greenish yellow, green, greenish blue, and bluish violet were obtained. The variations in color were quite distinct from pH 4.0 to 8.0 but less vivid from pH 8.0 to 9.2.

Soil samples, the pH values of which had been determined by the electrometric method,[1] were used to test the reliability of the indicator. To 0.9 gm. of soil and 0.3 gm. of pure neutral powdered barium sulfate in Pyrex test tubes, 5 drops of indicator and 5 cc. of distilled water were added. The mixture was well shaken and left to stand overnight. Next morning the clear colored solutions were compared with the standards. The results are shown in table 1.

It will be observed that with the exception of soils 1376, 1377, and 1368, the results of the two methods agreed very closely. About 60 soil samples[2] from the

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[1] Kindly supplied by Y. Hseung, of the National Geological Survey of China.
[2] Collected by S. M. Chen.

tea plantations throughout Meitan were tested by means of this method. The pH values were found to lie between 4.0 and 6.0. These results correspond to the degree of acidity of ordinary tea soils recorded by other workers.[3]

TABLE 1

Comparison of pH values of soils as determined by the electrometric and mixed indicator methods

SOIL NUMBER	KIND OF SOIL	DEPTH OF SAMPLE	pH VALUES	
			Electrometric method	New mixed indicator method
		cm.		
1331	Alkali soil	0–10	9.34	9.2
1333	Alkali soil	30–40	9.33	9.2
2220	Light chestnut earth	0–35	8.40	8.6
2227	Dark chestnut earth	0–5	7.53	7.8
2228	Dark chestnut earth	5–36	7.97	8.0
1670	Wet soils	34–61	7.09	7.0
1671	Wet soils	61–76	7.07	6.9
2291	Yellow earth	32–51	7.12	7.2
2290	Yellow earth	0–32	6.85	6.8
1376	Podzolized brown earth	0–15	4.58	6.0
1377	Podzolized brown earth	15–40	4.86	5.6
1368	Podzolized brown earth	19–100	5.00	6.1

TABLE 2

Comparison of pH values of soils as determined by the electrometric, Truog, and powdery indicator methods

SOIL NUMBER	pH VALUES		
	Electrometric method	Truog method	Powdery indicator method
1331	9.34	8.4	8.8
1332	9.45	8.4	9.2
1333	9.33	8.4	9.2
2220	8.40	8.3	8.2
2227	7.53	8.2	7.5
2228	7.97	8.3	8.2
1670	7.09	7.5	7.1
1671	7.07	7.3	6.8
2290	6.85	8.0	7.0
2291	7.12	8.3	7.2
2292	6.80	8.2	7.4
2293	6.62	8.1	7.2
1368	5.00	5.0	5.3
1376	4.58	5.5	4.8
1377	4.86	4.6	4.6

PREPARATION AND USE OF THE POWDERY INDICATOR

The foregoing method, though simple and accurate, is not suitable for field work. An endeavor was made, therefore, to effect further improvement. It

[3] Details of this investigation are to be published elsewhere.

was found that when a concentrated solution of the new indicator was mixed with pure neutral barium sulfate, adjusted to pH 6.8, evaporated to dryness on the steam bath, and ground to pass a 100-mesh sieve, a bluish green powder was obtained.   When this powder was dusted on a drop of buffered solution of a certain pH value on a white porcelain plate, the characteristic color was produced. When it was dusted on moist soil surfaces, however, the color developed did not last long enough.   In order to fix the indicator on the barium sulfate, a dilute collodion solution was added to the mixture, which was then dried and reground. The powdery mixture was then ready for use.

The procedure adopted was as follows: About 0.5 gm. of soil was placed in a porcelain evaporating dish or, when several samples were to be tested at the same time, in the depressions of porcelain plates such as those used in the Truog set, moistened with about 1 cc. of distilled water, and stirred well with a glass rod.   By means of the tip of the glass rod, a drop or two of the soil suspension was transferred to a white porcelain plate and spread evenly so that a film about 1 cm. square and 0.5 mm. thick was formed.   Then the powdery indicator, contained in a vial capped with a 100-mesh brass screen, was carefully dusted on the moist film of soil suspension so that just the color of the soil was covered and no more.   About 0.01 gm. was enough for each determination.   After 5 minutes the color developed was compared with a painted color standard.   Results with this method are presented in table 2, in which are also given for comparison, the results obtained by the Truog method.

These results demonstrated that agreement between the new method and the electrometric method was good for all soils except 2292 and 2293, and in general it was better than that obtained by the Truog method.   The Truog method failed badly with soils 2290, 2291, 2292, and 2293.   The fact that all these soils belong to the Yellow Earth group seems worthy of serious consideration.   The Truog method, however, should not be blamed for disagreement between numbers 1331, 1332, and 1333, since the upper limit of alkalinity shown by its color standard is pH 8.5.

CONCLUSIONS

It is urgent to have all agricultural soils in China tested so that adjustments of their reactions can be made, to favor the growth and increase the yield of crops. As the importation of foreign testing sets is difficult and expensive, the proposed powdery indicator method meets the demand in a timely fashion.   This method is recommended for its simplicity, rapidity, and reasonable accuracy.   The powdery form of the reagent facilitates carriage and storage, a great advantage over the solution form.   The new method should also find wide application in various industries.   The reactions of turbid and colored solutions, falling within the effective pH range of this method but to which colorimetric work is inapplicable, could be conveniently and speedily determined

COMPARISON OF BASE-EXCHANGE EQUATIONS FOUNDED ON THE LAW OF MASS ACTION[1]

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U. S. Department of Agriculture

Ordinary soils which usually contain a considerable proportion of replaceable calcium are converted to alkali soils with exchange of calcium for sodium. Chemical reclamation of such soils depends on the reverse reaction. It is believed that these reactions follow chemical laws and that definite equilibriums exist between the proportion of each cation on the exchange complex and the concentration of these cations in the soil solution. With a knowledge of these equilibriums and the composition of the corresponding soil solution, one could estimate the amount of each cation on the exchange complex in equilibrium with it. Also one could estimate the maximum absorption of the various cations on a soil which could be obtained by a prolonged leaching with a solution of known composition and concentration.

Investigators sometimes have had difficulty in fitting base-exchange equations to experimental data involving both monovalent and divalent ions: first, because in many such reactions a shift in equilibrium has occurred upon dilution of the of the system, and second, because the same equilibrium is not always obtained when approached from either direction.

This paper presents data on the equilibriums obtained when calcium-saturated soils are treated with sodium and ammonium chloride solutions.

LITERATURE REVIEW

Base-exchange equations can be divided into two general types: (*a*) those which are empirical and are related to an adsorption isotherm, and (*b*) those which are based on the mass-action law or some modification of it. Wiegner (20) was one of the first to note that in certain base-exchange reactions a shift in equilibrium occurred with dilution of the system. This shift apparently occurs only when exchange takes place between ions of dissimilar valence. Renold (12) asserts that it is generally absent in cases of monovalent exchange, whereas it has frequently been observed in exchange involving dissimilar valence. In the same article he ascribes the shift in equilibrium with dilution to the presence of heavy metal ions.

Kerr (8) applied the mass-action equation to exchange between calcium and hydrogen ions. For simplicity, exchange pairs of this nature are herein desig-

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nated as Ca/H where calcium is the original cation present. Kerr wrote his base exchange equations as follows:

$$CaX + 2HCl \rightleftharpoons CaCl_2 + H_2X \qquad (1)$$
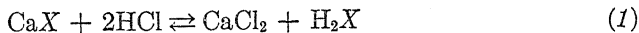
and on this basis the equilibrium equation becomes

$$\frac{(Ca^{++})(H_2X)}{(H^+)^2(CaX)} = K \qquad (2)$$

in which X represents the absorbing complex of the soil.

In a later paper (9) he reported on Ca/NH$_4$ exchange and, on the basis that the soil acid was monobasic, wrote his equilibrium equation as follows:

$$\frac{(NH_4^+)^2(CaX_2)}{(NH_4X)^2(Ca^{++})} = K \qquad (3)$$

Kerr did not vary the amounts of soil or the total volume in his experiments and obtained fairly uniform equilibrium constants.

In 1932, Vanselow (19) studied the Ca/NH$_4$ and NH$_4$/Ca reactions, varying both concentration and amount of exchangeable material. In the latter case, Kerr's equation failed to give constant values. Vanselow attributed this to the formation of mixed crystals in the absorption complex, and substituted in Kerr's equation mol fractions to represent CaX$_2$ and NH$_4$X. On this basis his equation for Ca/NH$_4$ exchange became:

$$\frac{Ca^{++}}{(NH_4^+)^2} \times \frac{(NH_4X)^2}{CaX_2(CaX_2 + NH_4X)} = K \qquad (4)$$

When the amount of soil was kept constant in the series, Kerr's and Vanselow's equations fit about equally well, but when the amount of absorbing material was varied, Vanselow's equation gave the best fit.

Gapon (2), in Russia, developed a new "mass-action" equation which has only recently come to the attention of American workers. He represents exchange by the equation:

$$M_1X + M_2 \rightleftharpoons M_2X + M_1$$
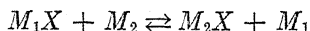
where X represents the base-exchange complex, and M_1 and M_2 represent two kinds of cations. At equilibrium, V_1, the velocity to the right, equals V_2, the velocity to the left. But the velocity V_1 is proportional to the surface occupied by the molecules M_1, which we shall designate by F_1, and the concentration of molecules M_2, which we shall designate as C_2. Then

$$V_1 = K_1F_1C_2$$

and similarly

$$V_2 = K_2F_2C_1.$$

The equilibrium constant K is then equal to $\dfrac{F_2C_1}{F_1C_2}$.

For Ca/NH_4 base exchange, Gapon writes the equation as follows:

$$Ca_{\frac{1}{2}}X + NH_4 + NH_4X + \tfrac{1}{2}Ca^{++} \tag{5}$$
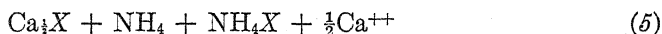
He points out that on an absorbing surface, calcium behaves as does sodium when expressed on a milliequivalent basis which leads to the equation:

$$\frac{Ca^{++}}{NH_4^+} \times \frac{NH_4X}{CaX} = K \tag{6}$$

It should be noted that in this equation, C_1 and C_2 are given in millimols per unit volume and NH_4X and CaX in milliequivalents.

Jenny (7) later developed a kinetic theory of base exchange for ions of equal valency founded on statistical considerations. Hofmann and Giese (6) adapted this equation for Ca/NH_4 exchange and obtained:

$$\frac{(NH_4^+)^2}{Ca^{++}} \times \frac{(S - NH_4X)}{V(NH_4X)^2} = K \tag{7}$$

This equation is the same as that of Kerr (3) except that, according to our conventions, it is written as being approached from the right and it contains a volume factor. V here represents the volume corresponding to a certain amount of soil. These authors appear to be the first to introduce a volume factor in mass action equations for base exchange.

Ogg (10) made a study of the Ca/NH_4 reaction on Iowa soils and tried to fit the results into the straight line forms of Vageler's (17, 18) and of Gapon's equations. He asserted that the soil-water ratio changed the slope of his curves, and found the Vageler equation superior in expressing exchange between H^+ and Ca^{++} or Na^+. The Vageler equation is empirical and based on amounts of reactants at the beginning of the reaction.

In recent years it has been shown that inorganic base-exchange materials in soils are primarily mineral in nature. [See Grim (4) for a good summary review.] It is to be expected that with different kinds of minerals and chemical or physical bonds, equilibriums are different in different soils, depending on the kind and relative amounts of the minerals present and the ion-exchange pairs under consideration. Thus Schachtschabel (15), and Hendricks and Alexander (5) have shown that from a mixture of calcium and ammonium salts, montmorillonites will take up relatively more calcium than will micaceous minerals, kaolin, muscovite, or biotite.

MATERIALS AND METHODS

Calcium-saturated Fallbrook sandy loam was used in these experiments. The Fallbrook series are primary soils derived from granites, are reddish brown in color and mildly alkaline in reaction, but without free lime (1). These soils are formed under climatic conditions of 12 to 20 inches of rainfall during the warm winters, and of hot dry summers tempered to some extent by occasional

high fogs and haze. Hendricks,[3] using both x-ray diffraction and thermal analyses methods, found the colloid of this soil to be mainly montmorillonitic. The whole soil was used in these experiments. It contained 0.8 per cent organic carbon. The soil was saturated with calcium by leaching with 0.5 N calcium chloride, washing with water, and finally with 90 per cent alcohol.

These investigations involved the preparation of equilibrium soil-water suspensions, the determination of the bases in the equilibrium extracts, and the removal and determination of the exchangeable bases of the soil.

The equilibrium suspensions were prepared by shaking a bottle containing a given weight of calcium-saturated soil with the desired salt solution for 1 hour in a reciprocal shaker. The suspensions were allowed to stand overnight, and then were filtered by means of a Büchner funnel and a paper filter, or a carbon filter funnel and absorbent cotton, depending on the amount of soil in the suspension. All of the filtrates were analyzed for calcium and sodium and, in addition, a few were analyzed for magnesium, potassium, and manganese.

For the determination of the exchangeable bases the soil on the funnel was washed free of soluble salts with 95 per cent alcohol, transferred to a flask and shaken with N NH$_4$Ac, pH = 7.0. The suspension was filtered on cotton in a carbon filter funnel. More ammonium acetate was passed through the soil until 500 cc. of solution was collected. Suction was used when necessary to give an approximate rate of leaching of 1 ml. per minute. The leachates were analyzed for calcium and sodium, and occasionally for magnesium, potassium, and manganese.

The calcium, magnesium, and potassium were determined by the semimicro-methods of Reitemeier (11). Sodium was determined according to Wilcox.[4] The interference of phosphate was eliminated by obtaining the water-soluble precipitate of sodium by difference.

RESULTS AND DISCUSSION

The principal factors governing equilibrium in base-exchange are ratios of the competing ions in the solution, volume, size of soil sample, concentration, and temperature. These factors were studied each in turn.

Effect of sodium percentage of solution

Data are presented in table 1 for equilibrium between 100 gm. of Fallbrook sandy loam and solutions of various initial sodium percentages.

The equilibrium constant calculated according to equation (2) appears in column 12 of table 1 and is obtained as a product of columns 5 and 10. Column 13 is obtained by dividing the value in column 12 by the sum of NaX + CaX$_2$ in millimols, and gives the equilibrium constant according to Vanselow. To obtain Gapon's constant, the authors first calculated column 6, using millimols

[3] Personal communication, January 17, 1944.

[4] Wilcox, L. V. 1941 Methods of analyses used in the Rubidoux Laboratory, Riverside, California. Mimeographed, 3rd ed. U. S. Dept. Agr.

rather than mols, and column 11, using milliequivalents in place of equivalents. Then the product of columns 6 and 11 gives column 14.

The mass-action equation is based on the active masses of reactants, hence activity coefficients should be used. Test calculations showed that the use of activity coefficients, changed the value of the equilibrium constants in tables 1 and 2 very little, hence concentrations were used directly in these tables.

A study of the data in columns 12, 13, and 14 indicates that Gapon's equation gives the best fit, with a coefficient of variation of 2.7 compared to 12.4 and 10.9 for Kerr's and Vanselow's equations respectively.

TABLE 1

Equilibrium data for 100 gm. Fallbrook sandy loam treated with waters of varying sodium percentage and 10 m.e./l. total salt content

Volume 1 liter

Na ORIGINAL SOLUTION	EQUILIBRIUM SOLUTION					EXCHANGEABLE BASES					EQUILIBRIUM CONSTANT K		
	Na	Ca	Ca	$\dfrac{Ca}{Na_2}$	$\dfrac{\sqrt{Ca}}{Na}$	Na	Ca	Ca	$\dfrac{NaX^2}{CaX}$	$\dfrac{NaX}{CaX}$	Kerr	Vanselow	Gapon
1	2	3	4	5	6	7	8	9	10	11	12	13	14
per cent	$\frac{m.e./}{l.}$	$\frac{m.e./}{l.}$	$\frac{mmol./}{l.}$			*m.e.*	*m.e.*	*mmol.*					
50	4.70	6.44	3.22	.1459	.382	.34	13.0	6.50	.0178	.0261	.00260	.000380	.0099
80	7.43	3.56	1.78	.0324	.180	.64	12.2	6.10	.0671	.0525	.00217	.000320	.0095
90	8.28	3.17	1.59	.0231	.152	.75	12.0	6.00	.0937	.0625	.00216	.000321	.0095
95	8.80	2.59	1.30	.0169	.130	.85	11.7	5.85	.123	.0725	.00208	.000310	.0094
98	9.02	2.01	1.01	.0125	.112	.93	11.6	5.80	.149	.0801	.00186	.000275	.0090
100	9.12	1.97	0.99	.0119	.109	.92	11.1	5.50	.153	.0829	.00182	.000281	.0090
Mean											.00211	.000314	.00938
Coefficient of variation											12.4	10.9	2.7

Effect of varying volume and of dilution

In the next trial 25 gm. samples of Fallbrook sandy loam were treated with varying volumes of a solution containing 27 m.e. NaCl and 3 m.e. CaCl$_2$ per liter. The data are presented in table 2.

Examination of the data in columns 12, 13, and 14 in table 2, indicates that here also Gapon's equation fits the experimental data more closely than do the other two.

Effect of varying amount of soil

In a third test, the amount of soil was varied, and these data are presented in part B of table 2. It will be seen that here Gapon's equation has the lowest coefficient of variation, while Kerr's equation gives values that do not have any semblance of constancy. If, however, the values given in column 12 are multiplied by the appropriate factors so that the results are all on the basis of equal amounts of soil, relative constancy is obtained for Kerr's equation also.

Thus, if the first value .000074 is multiplied by 20 so as to give results on the basis of 100 gm. of soil, the result is .00148, which agrees fairly well with the value .00135 for the case where 100 gm. of soil was used initially. It is believed that the failure to recognize the necessity of comparing results on equal amounts of soil has led to unsatisfactory results with Kerr's equation in the hands of several investigators. It is now clear that the equation of Hofmann and Giese (7) is

TABLE 2

Equilibrium data for Fallbrook sandy loam and solutions containing 27 m.e./l. NaCl + 3 m.e./l. CaCl₂

VOLUME OR WEIGHT	EQUILIBRIUM SOLUTION					EXCHANGEABLE BASES					EQUILIBRIUM CONSTANT K		
	Na	Ca	Ca	$\frac{Ca}{Na^2}$	$\frac{\sqrt{Ca}}{Na}$	Na	Ca	Ca	$\frac{NaX^2}{CaX}$	$\frac{NaX}{CaX}$	Kerr	Vanselow	Gapon
1	2	3	4	5	6	7	8	9	10	11	12	13	14
	m.e./l.	m.e./l.	mmol./l.			m.e.	m.e.	mmol.					
A. Volume varied, 25 gm. soil													
ml.													
25	19.38	13.42	6.71	.0180	.134	.22	3.72	1.86	.026	.059	.00047	.00023	.0079
50	21.80	10.40	5.20	.0109	.104	.27	3.56	1.78	.041	.076	.00045	.00022	.0079
125	23.9	7.23	3.62	.0063	.80	.33	3.43	1.72	.062	.096	.00039	.00022	.0077
500	24.6	4.72	2.36	.0039	.062	.44	3.07	1.54	.125	.143	.00049	.00025	.0089
1000	26.8	4.12	2.06	.0029	.054	.48	3.10	1.55	.149	.155	.00043	.00021	.0084
2500	26.9	3.71	1.86	.0026	.051	.52	3.11	1.56	.173	.167	.00045	.00021	.0085
Mean											.000447	.000223	.00822
Coefficient of variation											32.4	6.4	5.6
B. Amount of soil varied, volume 500 ml.													
gm.													
5	26.9	3.78	1.89	.0026	.051	.089	.567	.28	.0283	.157	.000074	.00020	.0080
15	26.4	4.15	2.08	.0030	.055	.272	1.92	.96	.077	.142	.00023	.00019	.0078
50	25.6	5.45	2.73	.0042	.065	.777	6.65	3.33	.181	.117	.00076	.00018	.0076
100	24.35	6.76	3.38	.0057	.075	1.26	13.4	6.70	.237	.094	.00135	.00017	.0071
Mean												.000185	.0076
Coefficient of variation												7.0	5.1

very similar to Kerr's equation, indicating that data should be compared on the basis of equal base-exchange capacity.

It will be noted that the constants in column 14 of tables 1 and 2, agree well within each table. They do not agree so well between tables. This is attributed to small changes in the manner of washing the soil and in determining the exchangeable bases. No attempt was made to conduct the determination at constant temperature. Room temperature during these investigations varied from about 20° to 30°C. Critics will also point out that there are many places in tables 1 and 2 where exchange apparently was not stoichiometric.

In these studies and others conducted simultaneously, there was clear evi-

dence that appreciable amounts of bases were dissolved from soil minerals and that these bases, once in solution, influenced equilibriums. Thus, a sodium-saturated soil, mixed well with a liter of distilled water and allowed to stand some time, was found on analysis to contain a relatively large amount of replaceable calcium and some replaceable potassium. It is believed that the analyses reported in tables 1 and 2 are more accurate than those customarily reported in equilibrium studies where, for instance, a calcium-saturated soil may be shaken with a solution of ammonium chloride, only the solution analyzed, and equilibrium data calculated on the basis of stoichiometric exchange, no solution of minerals being assumed.

TABLE 3

Equilibrium data from Gedroiz, reported by Gapon, on 10 gm. Tula chernozem soil plus 100 ml.
NH₄Cl of varying concentration

Results calculated to the basis of 100 gm. soil

NORMAL-ITY	NH₄	γNH₄	Ca	γCa	$\frac{\gamma Ca}{(\gamma NH_4)^2}$	$\frac{\sqrt{\gamma Ca}}{\gamma NH_4}$	NH₄	Ca	$\frac{(NH_4X)^2}{CaX}$	$\frac{NH_4X}{CaX}$	KERR	VAN-SELOW	GAPON
1	2	3	4	5	6	7	8	9	10	11	12	13	14
	m.e./l.		*mmol./l.*				*m.e.*	*m.e.*					
.01	7.1	.874*	1.45	.802‡	.030	.174	2.9	34.1	0.49	0.085	.0147	.0074	.0148
.05	41.2	.786*	4.40	.677‡	.0029	.053	8.8	28.2	5.5	0.312	.0154	.0067	.0165
.10	86.9	.738*	6.55	.613‡	.00098	.0312	13.1	23.9	14.3	0.548	.0140	.0040	.0171
.50	474.9	.617*	12.55	.496§	.000072	.0085	25.1	11.9	106.0	2.11	.0076	.0024	.0179
1.00	971.2	.572*	14.40	.461§	.000022	.00464	28.8	8.2	201.0	3.51	.0044	.0013	.0163
2.00	1969.7	.548†	15.15	.467§	.0000061	.00247	30.3	6.7	273.0	4.52	.0017	.0005	.0112
4.00	3967.4	.554†	16.30	.557§	.0000019	.00137	32.6	4.4	483.0	7.41	.0009	.0003	.0102

* Calculated from data of Scatchard and Prentiss (14), without correction for temperature.

† Values from KCl from Robinson and Horned (13) divided by 1.05.

‡ From Shedlowsky and MacInnes (16).

§ From Robinson and Horned (13).

Effect of solution concentration and dilution

In tables 1 and 2 the soil at equilibrium was less than 10 per cent saturated with sodium. It has been suggested that a truer picture of equilibrium would be indicated if a larger portion of the original calcium-exchange capacity were replaced by a new ion. This can be shown by reproducing as table 3 a table from Gapon, who used analytical data from Gedroiz (3).

In the preparation of table 3, activity coefficients were used. Thus the ionic strengths of the solutions were first calculated, and activities of ammonium chloride at the lower concentrations calculated from the data of Scatchard and Prentiss (14). No data were available for activity coefficients of ammonium chloride above 1 N; hence, values for potassium chloride divided by 1.05, which is the ratio of the activity coefficients of KCl and NH₄Cl at a concentration of 1 N, were used. Gapon obtained good agreement by using his equation without correcting for activity up to concentrations of 1 N. He said that he believed agreement would be improved above this concentration if activity coefficients

were used. The data given in column 14 of table 3 indicate that agreement is fair even to 4.0 N if activity coefficients were used. The equilibrium constants in table 3 have been calculated on the basis of milliequivalents and millimols as in tables 1 and 2, rather than as equivalents and mols as was done by Gapon.

In these tests in a 1 N solution the soil was 78 per cent saturated with ammonium, and analytical difficulties in washing and determining NH_4X may well have entered. It will be noticed that over this concentration range the equation of Kerr does not fit well, and that of Vanselow is even less satisfactory.

Tests with a number of other western soils have been made, some of which were highly calcareous, and in each case, the equilibrium equations, particularly that of Gapon, agreed well with the experimental data.

From equations (3) and (4) it is apparent that the driving force is $\dfrac{Ca^{++}}{(NH_4^+)^2}$, whereas in equation (6) it is $\dfrac{\sqrt{Ca^{++}}}{NH_4^+}$. If the concentrations of calcium and ammonium ions are 10 m.e. per liter each, the value of the fraction according to equations (3) and (4) is 10/100 or 0.1. If these solutions are now diluted to one-half their original concentrations, the values are 5/25 or 0.2. Thus the magnitude of the driving force changes with dilution. In the case of equation (6) dilution also causes a change in the driving force. It is evident, then, that equations (3), (4), and (6) explain quantitatively the effects obtained by dilution in base-exchange reactions, whereas the empirical equations based on the adsorption isotherm do not. It is also apparent that dilution effects appear only when cations of dissimilar valence are present.

Effect of temperature

Wiegner (20) reported that the effect of temperature on Ca/NH₄ equilibrium in permutits was small, with a slight decrease in NH₄ absorbed with increases in temperature. He found that an average of 6.554 millimols NH₄ were absorbed at 50.4°C., compared to 6.813 millimols at 5.1°C. Vanselow (19) gives data indicating similar results for Ca/NH₄ exchange in bentonite. The authors determined the equilibrium constant for Ca/Na exchange on another soil and obtained .00976, .00982, and .00992 at 7°C. versus .00878, .00933, and .00962 at 30°C. This is in the same direction but smaller in magnitude than that found by Venselow for Ca/NH₄ exchange. The change in equilibrium constant for ranges in temperatures found in laboratories is therefore of the same order as ordinarily encountered experimental errors.

SUMMARY

Fallbrook sandy loam saturated with calcium was treated with mixtures of sodium chloride and calcium chloride in which sodium concentration and volume and amount of soil were each varied in turn. The equilibrium data were then examined to test the closeness of fit with the base-exchange equations of Kerr, Vanselow, and Gapon. The equation of Gapon gave the best fit. This was also found to be the case when analytical data, reported by Gapon, were examined.

The shift in equilibrium with dilution is satisfactorily explained by each of these three equations, but not by the absorption type equations. At increased temperatures, slightly smaller amounts of sodium are present in the soil at equilibrium.

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PERMEABILITY-CAPILLARY POTENTIAL CURVES FOR THREE PRAIRIE SOILS[1]

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The flow of liquids in unsaturated porous media has been the subject of recent study. Buckingham (3), Gardner (4), Richards (7), and Richards and Wilson (10) have developed the general theory and set up the general equations of flow, and Richards (7) has solved the general equation of flow under steady-state conditions for the linear case using an experimentally determined expression relating permeability and capillary potential. To solve the general flow equation, $-\dfrac{\partial \rho}{\partial t} = \nabla \cdot (\rho V)$, V, the macroscopic velocity, must be eliminated. The analytical difficulties encountered in finding a general expression for the velocity of flow in soil moisture films appear insurmountable, therefore $V = -K\rho\nabla\Phi$, Darcy's law, is used. ρ is the density of the liquid, K the permeability, and $\nabla \Phi$ the total liquid moving field. Gardner (4) and Bodman (1) assume that in unsaturated media the liquid moving field may be analytically expressed by the relation $\nabla \Phi = \nabla (\psi + \phi + \lambda)$, where ψ is the capillary potential, expressed in ergs per gram, due to the tension within the fluid caused by the surface tension and the curvature of the air-liquid interface. This interfacial curvature is dependent upon the moisture content of the medium. ϕ is the body force due to gravity, and λ is the osmotic pressure arising from the substances in solution in the soil water. In this article the osmotic pressure will be considered constant, as it is assumed that the concentration of the soluble substances is constant throughout and therefore $\nabla \lambda = 0$.

Some objections have been made to the use of Darcy's equation in the field of unsaturated flow where the equation has neither been established theoretically nor shown to hold experimentally. In unsaturated flow, however, the unsaturated permeability is not a constant for a given medium but varies with the capillary potential of the soil moisture as well as with the different media and must be determined experimentally at different capillary potentials. Hence, any deviation from the assumed law would be included in the permeability function K, and the consequent solutions of the flow equation should give correct values.

Richards (7), and Richards and Wilson (10) obtained permeability-capillary potential relations for soils of disturbed structure. The experiments discussed in the present paper were performed to obtain the permeability-capillary poten-

tial relations for other soils and to compare the permeability of soil having disturbed structure with that of the same soil having natural field structure.
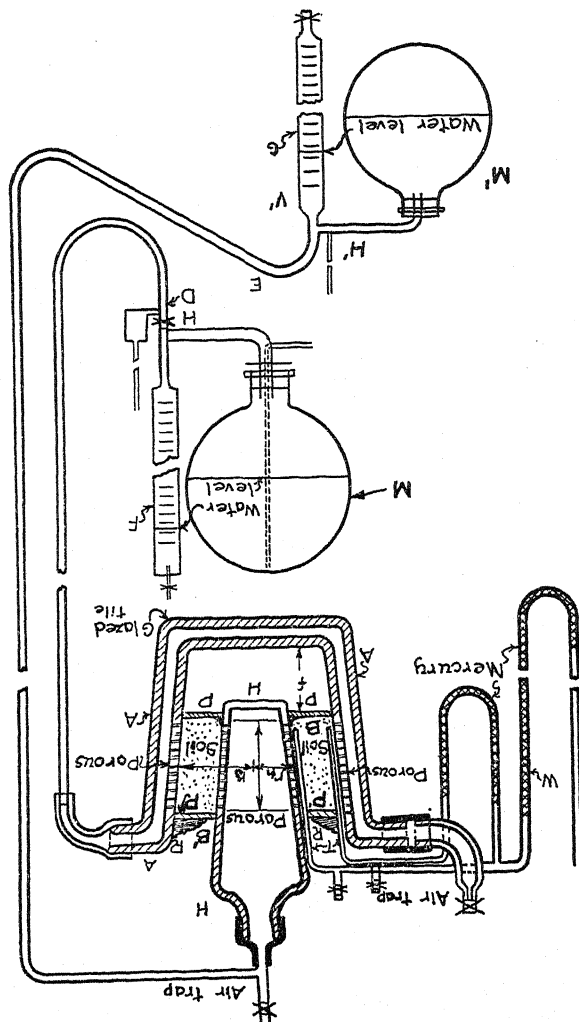
FIG. 1. AUTO-IRRIGATOR APPARATUS FOR MEASURING UNSATURATED PERMEABILITY OF SOILS WITH DISTURBED STRUCTURE

MATERIALS AND METHODS

Radial flow

Radial flow units were constructed to measure the capillary flow rate and the pressure gradient in samples of Marshall and Shelby silt loams and of Dickinson fine sand with disturbed and undisturbed structures. The apparatus used in making these measurements is shown in figure 1. The inlet cell for the radial

flow unit was a double-walled auto-irrigator pot, A, similar to that described by Richards and Blood (9). A metal plate, P, was supported f cm. from the bottom of the pot. This plate fitted tightly against the inside wall of the pot. Through a centered hole in the plate a tapered porous cup, H, which served as the outlet, was suspended. The diameter of the hole was about 0.3 cm. larger than the diameter of the suspended cup. A tightly fitting rubber gasket, B, overlapping the plate, was placed about the cup and prevented the soil from reaching the lower part of the pot. A layer of soil, supported by the metal plate, was placed between the pot and the outlet cup; this soil layer was covered by another metal plate P' and gasket B' fitted as were P and B. On the outside and inside of the upper plate, holes were drilled and tensiometer tubes, T, 0.5 cm. in diameter and 6 cm. long, were inserted into the soil against the walls of the pot and cup. The entire top was sealed with paraffin wax in such a manner that the porous cup was free to settle tightly against the soil. The inner tensiometer tube was connected to a mercury manometer W. A differential mercury manometer was connected between the two tensiometer tubes. A small hole in the upper gasket allowed the soil air to remain at atmospheric pressure. To provide water under constant tension at the inlet, a long rubber tube connected the inlet to flask D. Operating on the Mariotte flask principle, this flask was connected to the burette F, and storage reservoir M. The lower cell was connected to the overflow level E, and this to the measuring burette and storage reservoir M'. When tensions greater than those which could be obtained by lowering the inlet and outlet were desired, the apparatus was connected to a vacuum barostat apparatus described by Richards (8).

The permeability will be considered constant for each setting to facilitate the solution of the equation, since the tension in the water throughout the soil for any given setting is almost constant, the difference in tensions between the inlet and outlet being kept small. The permeability is plotted against the average tension (negative pressure) at the inlet and the outlet.

If Q/t is the inflow in cubic centimeters per second, r_h and r_a are the radii of the pot and cup respectively, L the length of the soil ring, p the pressure difference between the inlet and outlet walls, then rate of flow, pressure difference, inlet and outlet radii, and the permeability are related by the equation (6, p. 152):

$$K = \frac{Q}{t} \frac{\ln \frac{r_h}{r_a}}{2\pi L p} = \frac{Q}{t} \frac{\ln \frac{r_h}{r_a}}{2\pi L \psi} \qquad (1)$$

since capillary potential $\psi = \int \frac{dp}{\rho}$ and ψ is numerically equal to pressure p when the density of the water is constant and equal to unity.

If the boundary conditions of this radial flow system are characterized by

$$r = r_a \qquad \psi = \psi_a$$

$$r = r_h \qquad \psi = \psi_h \qquad (2)$$

where ψ_h is the capillary potential at the soil pot wall and ψ_a is the capillary potential at the outlet cup wall.

The relationship between the capillary potential and the radius r is found to be (6, p. 152)

$$\psi = \left(\frac{\psi_h - \psi_a}{\ln \dfrac{r_h}{r_a}}\right) \ln r + \frac{\psi_h \ln r_a - \psi_a \ln r_h}{\ln \dfrac{r_a}{r_h}} \tag{3}$$

Cylinder method: soil with natural field structure

To determine the permeability of soils having natural field structure, other arrangements of porous plate apparatus were used. One was similar to that described by Richards (7) except that a circular cell was used. The soil column, approximately 25 cm. in diameter and 16 cm. long, was obtained by jacking a galvanized cylinder into the undisturbed soil.

To ensure good contact, the upper cell was held firmly against the soil column by strong rubber bands. To prevent loss of moisture from the system, the telescoping frame was wrapped with impervious adhesive rubber and each cell was tightly sealed to the frame with roofing tar. A small hole in the upper seal allowed the soil air to remain at atmospheric pressure.

Before the system was put into operation the cells were filled with boiled distilled water and the entire system was wetted slowly at reduced pressure. The pressure in the soil water at each end of the soil column was measured by tensiometers connected as described in the preceding section. Data was obtained from two units. Frame 1 was filled with undisturbed Marshall silt loam taken from the Soil Conservation Experiment Farm near Clarinda, Iowa; and frame 2, with Shelby silt loam from near Bethany, Missouri. Soil samples containing holes or cracks were discarded.

In all the experiments, both radial and linear, the volume of water used was kept at a minimum. Distilled water was used to wet the soil. The soil percolate was used repeatedly so that the soluble substances would not be leached continually from the soil thus dispersing the colloids, altering the soil structure, and thereby changing the soil permeability. It was not possible to prevent a slow steady loss of moisture from the flow systems. This loss appeared to be independent of the flow rate or the capillary potential. It was assumed that equilibrium was reached when the flow rate into the system became constant and exceeded the outflow rate by a small fixed amount not exceeding 0.05 cc. daily for any setup and the soil moisture tension and tension differences remained constant. The flow rate was then determined, the capillary potential and potential difference were ascertained, and the permeability for the given capillary tension was computed. For the cylinder, if Q/t is the inflow rate in cubic centimeters per second, a, the area of the soil column, L, its length, and P, the pressure difference in dynes/cm.[2] between the ends of the soil column, then we may write Darcy's law for downward flow:

$$\frac{Q}{t} = -aK\rho \left(\frac{\nabla P}{\rho} + g\right) \tag{4}$$

and solving for K we obtain:

$$K = -\frac{Q}{t}\frac{1}{a\rho\left(\dfrac{\nabla P}{\rho} + g\right)} \qquad (5)$$

Large differences in pressure between the ends of the soil column were avoided, since the permeability as measured is an average for the range of values of the capillary potentials existing in the soil column. This average permeability is assumed to be equal to the actual permeability for the average capillary potential. Since permeability is not a linear function of the capillary potential the average permeability is not, in general, equal to the actual permeability when averaged over large differences in capillary potential.

Column method

Only one permeability value could be obtained at a given time for a given capillary potential in the conductivity measuring arrangements described above. Additional values were obtained by changing the soil moisture tension, allowing water to flow through the soil until steady-state flow was again attained before another permeability value could be determined. For soils, such as Marshall silt loam, which showed a marked decrease in saturated permeability with the flow of water through them, it is difficult to interpret the (K, ψ) curve. How much of the conductivity decrease is caused by soil structural changes, and how much by the change in capillary potential, cannot be determined. To obtain a (K, ψ) curve on which all points are determined at a given time, a flow apparatus similar to that described by Moore (5) was used.

A galvanized iron cylinder 25 cm. in diameter and 36 cm. long was forced into Marshall silt loam. A similar cylinder was filled with disturbed fine sand tamped to the desired porosity. In each cylinder, holes were punched 3, 10.5, 18, 25.5, and 33 cm. from the top of the cylinder. Tensiometer cups were inserted horizontally through these holes, and the cylinder-cup junctions were sealed to prevent loss of moisture. The cylinder containing the soil was set on a porous cell like that described in the preceding section. The system was wet under a vacuum, and the inlet was connected to a Mariotte flask similar to that previously described. The water from the inlet moved upward through the soil and evaporated from the upper layer of soil.

The rate of water uptake was measured. Tensions shown by the mercurial manometers connected to the tensiometer cups were frequently read. When readings of the tensiometers and the rate of water uptake became constant it was assumed that equilibrium had been reached. All experiments were carried out at 25° C. The (ψ, z) curve for these data were plotted for upward flow. The tangent, $\dfrac{\partial \psi}{\partial z}$, was determined from the curve and the conductivity calculated by means of Darcy's law modified for upward flow: $K = -\dfrac{Q}{t}\dfrac{1}{a\rho\left(\dfrac{\partial \psi}{\partial z}\Big/ \rho - g\right)}$

Saturated flow experiments

Preliminary laboratory experiments indicated that the permeability of soil at a given capillary potential was much less at the end of a flow cycle than at the same capillary potential at the beginning of such a cycle. In an unsaturated flow cycle, the soil is wet under a vacuum, and the permeability is determined for decreased moisture contents down to field capacity. The soil is then allowed to absorb moisture and the permeability measured for soil moisture contents up to near saturation. To compare the decrease in permeability for unsaturated flow with the change in permeability with time for saturated flow, saturated flow experiments were conducted.

Samples of Marshall silt loam having natural field structure were obtained and placed in a constant-head permeameter similar to that used by Stearns (11). The percolate flowed upward through the soil and was used repeatedly. The vertical macroscopic velocity of the water was small, never exceeding 0.07 cm. per minute. To compare the permeability and the time rate of change of permeability of disturbed soil with soil having natural field structure, similar experiments were performed with Marshall and Shelby soils which had been packed until the density of the soil with disturbed structure was equal to that of the soil with natural field structure. The variation of the permeability with time was observed for some of the soils for as long as 194 days.

RESULTS

Data from all of the unsaturated flow experiments are summarized graphically in figures 2, 3, and 4. In each figure the logarithm of the permeability is plotted as a function of the capillary potential. The curves obtained are similar to those of Richards (7) and Richards and Wilson (10). A study of the unsaturated flow curves shows that for the desorption and sorption curves for the same material, the sorption curve has appreciably smaller permeabilities for the same capillary potential than does the desorption curve. Likewise the saturated permeability of the soil shows a very marked decrease with time (fig. 5), as was shown by Bodman (2). The maximum permeability of the saturated soil was $51,200 \times 10^{11}$ $\dfrac{cc.}{sec. \ cm.^2 \left(\dfrac{dynes}{cc.} \right)}$ permeability units near the beginning of the experiment and decreased to about 60×10^{11} permeability units after continuous flow for 200 days for Marshall silt loam, whereas for Shelby silt loam the maximum saturated flow rate was $15,900 \times 10^{11}$ permeability units but the rate of decrease was much smaller than for the Marshall.

The maximum flow rates for nearly saturated soils were not so large as for the saturated, and at the largest capillary tensions the flow rate decreased to 1.5 permeability units. Upon rewetting, the permeability always was restored much nearer to its original value than was the case for saturated flow. In the experiments on capillary flow the time of flow and the quantity of water passing through each column of soil of like areas for the saturated and the unsaturated flow were nearly the same.

The decrease in saturated permeability of the soil with time may be attributed to one or more of the following: (a) air may come out of the solution and fill the pores; (b) the colloids in the soil may be dispersed and swell or be transported by the soil moisture; (c) material may be deposited from the solution, thereby cementing the openings; (d) the soil may be compacted during the flow; (e) the aggregates which determine the soil structure may be changed; (f) bacterial action may change the soil and pore space. For the unsaturated flow case the decrease in the permeability with decreasing capillary potential may be due to (a) the actual change in the permeability of the medium which was shown to occur

Legend:

Marshall silt loam Desorption   o
Marshall silt loam Sorption   ⊙
Shelby silt loam Desorption   □
Shelby silt loam Sorption   ▣
Dickinson fine sand Desorption   △
Dickinson fine sand Sorption   ▲

Log. (k × 10^{11})

Capillary Potential   ergs/gram
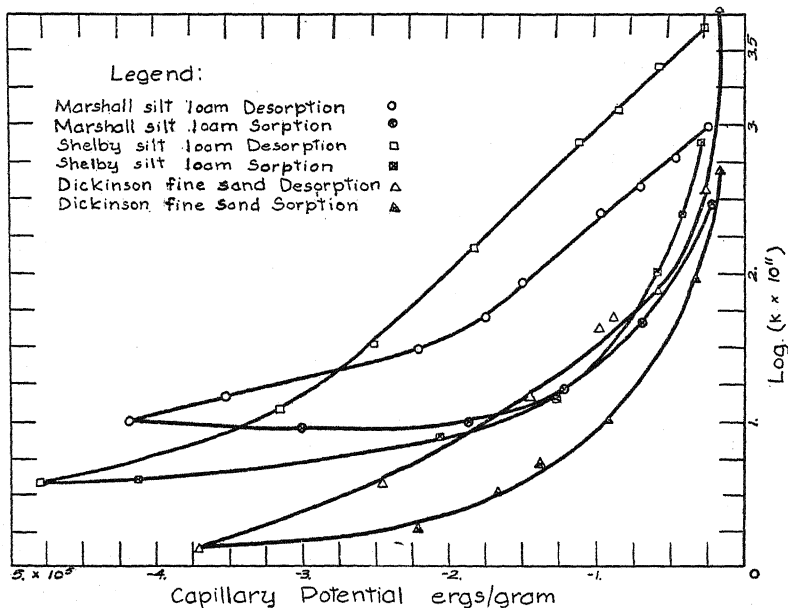
FIG. 2. LOGARITHM OF SOIL PERMEABILITY × 10^{11} AS A FUNCTION OF THE CAPILLARY POTENTIAL FOR DRYING AND WETTING OF SOIL WITH DISTURBED STRUCTURE OBTAINED BY THE AUTO-IRRIGATOR METHOD

in the saturated flow experiments, and (b) the decrease in the size and thickness of the moisture conducting films due to the drying of the soil. With an increase in the moisture content of the soil, the size of the moisture films should increase. Even if there is no actual change in the structure of the medium there is no reason to expect that the permeability at the same capillary potential should be equal during the desorption and sorption. Smith (12) has shown that for ideal soils a hysteresis effect should exist, since during desorption the size of the smallest throat of the assemblege determines the moisture content of the interstices, whereas, for sorption, the largest throat determines the moisture content of the interstices. Thus, the amount of water in the soil at a given capillary potential should depend upon its past history. This difference in the moisture content at a
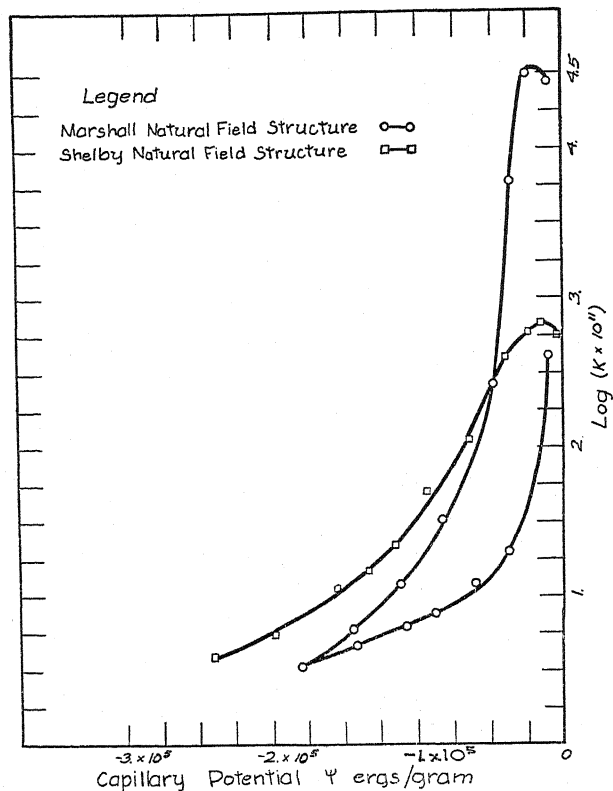
FIG. 3. LOGARITHM OF THE PERMEABILITY $\times 10^{11}$ AS A FUNCTION OF THE CAPILLARY POTENTIAL FOR DRYING AND WETTING OF SOIL WITH NATURAL FIELD STRUCTURE OBTAINED BY THE CYLINDER METHOD
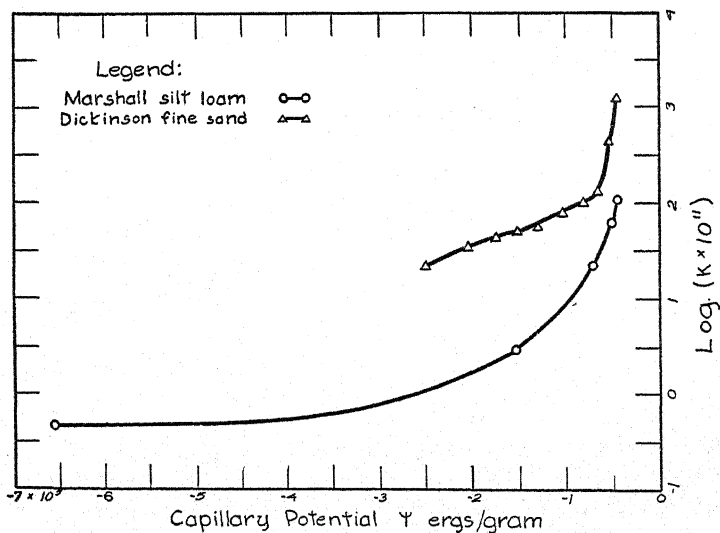
FIG. 4. LOGARITHM OF THE PERMEABILITY $\times 10^{11}$ FOR DESORPTION OBTAINED BY THE COLUMN METHOD

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given capillary potential may explain the decrease in the unsaturated permeability on the wetting curve as compared with that of the drying curve.

In figure 2 it is seen that the permeability of Marshall silt loam is about six times as great at a capillary potential of 0.5×10^5 ergs/gm. for the drying soil as for the soil being wetted. Likewise for Shelby silt loam the permeability is approximately ten times as great during desorption as when being wetted. Like differences are found for the disturbed Dickinson fine sand and the undisturbed Marshall and Shelby soils. However, the decrease in permeability for unsaturated flow for comparable times and amounts on going around a cycle is much less than the corresponding decrease in permeability for saturated flow for comparable times and amounts of water which flows.
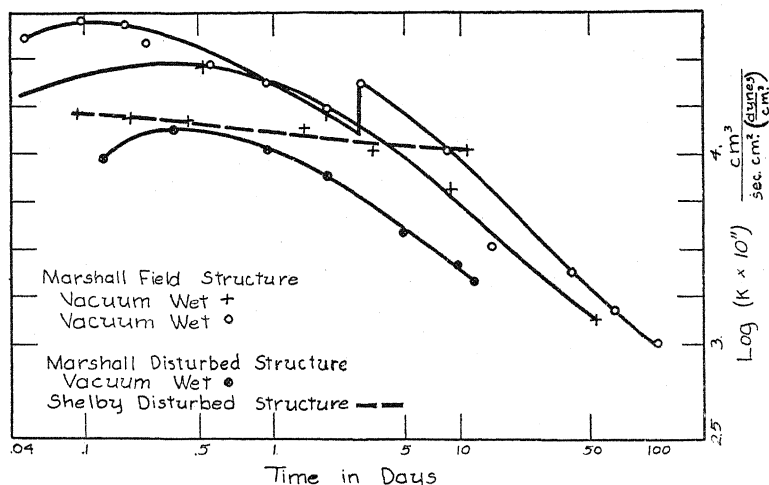
Fig. 5. Logarithm of the Saturated Permeability $\times 10^{11}$ as a Function of the Time in Days for the Flow of Moisture in Saturated Soil

A comparison of the permeability curves for soil of undisturbed structure, shown in figure 3, with the permeability for soil, of disturbed structure as shown in figure 2, indicates that the undisturbed Marshall soil has a much higher permeability when near saturation than does the soil with disturbed structure, but at higher capillary tensions the disturbed soil has the greater permeability. This may be explained by assuming that relatively large flow channels exist in the undisturbed soil and, when these are drained, fewer effective conducting channels exist than in the disturbed state where the soil structure was more uniform and the channels or moisture conducting films are smaller and therefore do not empty under so low a tension as for the undisturbed soil.

A study of figure 2 shows that the desorption permeability-capillary potential relation for Marshall and Shelby silt loams having disturbed structure may be analytically represented by the expression

$$\ln K = a\psi + b$$

over a limited range of moisture contents.

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(11) STEARNS, N. D. 1933 Laboratory tests on physical properties of water-bearing material. U. S. Geol. Survey Water-Supply Paper 596 F.

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PHYSICAL CHARACTERISTICS OF SOILS: VIII. STATE OF AGGREGATION

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The state of aggregation, or crumb structure, of a soil is determined by the degree to which the individual particles are cemented together in the form of compound particles. The cementing material is obviously colloidal matter, or clay. Although theoretically a soil could be made to have any state of aggregation by suitable treatment in the laboratory, natural soils do acquire a stable structure which, within limits, resists ordinary methods of cultivation. The exact mechanism of aggregate formation must be a complicated phenomenon in which the size of particles and the amount of clay are bound to play an important part.

The dominant role of the clay fraction in soil aggregation would be readily admitted. Exactly how it affects the formation of compound particles can be found by gradually removing this fraction and determining the state of aggregation of the soil at every step. Somewhat similar results would be expected if clay is gradually added to a colloid-deficient soil and the treated soil is mechanically analyzed on drying. Before the value of such experiments could be appreciated, however, it was necessary to test the reproducibility of results.

Three typical soils were selected for this purpose. These represented different types: P. C. 6, a laterite; P. C. 13, a black cotton soil; and Field Laboratory soil, a typical Punjab alluvium. These soils were repeatedly dispersed and dried and the dried samples mechanically analyzed by means of the chaino-hydrometer[1] and the Puri siltometer.[2] The method employed for dispersion consisted in shaking the soil suspension for 24 hours in a mechanical shaker with coarse sand. This method, the detailed description of which is given elsewhere[3], has the advantage that the soil is dispersed without involving its conversion into sodium soil. The result is that the dispersed soil on drying, unlike a sodium soil, will not redisperse in water, and its mechanical analysis reveals the state of aggregation exactly as this exists in the dry soil.

The results given in table 1 show that a dispersed soil when dried does acquire a stable and reproducible state of aggregation. Thus the influence of the removal of clay from or its addition to a soil could be studied with confidence. The same three soils were used for this purpose. They were dispersed by the

[1] Puri, A. N., and Puri, B. R. 1939 Physical characteristics of soils: IV. Density gradients in sedimenting columns and a chaino-hydrometer, for mechanical analysis of soils. *Soil Sci.* 48: 149–160.

[2] Puri, A. N. 1939 A siltometer for studying size distribution of silts and sands. *Punjab. Irrig. Res. Inst. Res. Pub.* 2(7).

[3] Puri, A. N., Dyal, P., and Rai, B. Studies in soil dispersion: I. Dispersion of soils by mechanical methods. To be published in *Indian Jour. Agr. Sci.*

sand method, and the clay fraction (<0.002 mm.) was gradually removed by repeated sedimentations and pourings, as in the beaker method of mechanical

TABLE 1

Reproducibility of results on repeated dispersion and drying of soils

SOIL NUMBER	DESCRIPTION	SUMMATION PERCENTAGES OF VARIOUS LIMITING DIAMETERS										
		0.002 mm.	0.005 mm.	0.01 mm.	0.02 mm.	0.06 mm.	0.10 mm.	0.20 mm.	0.30 mm.	0.40 mm.	0.50 mm.	0.60 mm.
P. C. 6	Dispersed and dried	6.0	6.8	10.8	25.3	58.2	67.5	84.0	95.0	99.0	100.0	
	Redispersed and dried	5.2	8.5	11.5	24.2	56.5	62.5	80.2	93.5	97.5	100.0	
	Dispersed again and dried	5.6	8.1	10.8	24.2	55.5	64.8	81.5	95.0	97.8	100.0	
F. L. Soil	Dispersed and dried	11.9	18.4	30.4	44.4	77.8	93.5	98.0	99.0	99.9	100.0	
	Redispersed and dried	10.8	18.0	30.1	43.8	75.5	92.5	97.5	99.0	99.6	100.0	
	Dispersed again and dried	10.8	17.1	29.5	43.8	76.2	92.2	94.5	98.2	99.0	100.0	
P.C.13	Dispersed and dried	1.4	5.3	9.7	19.1	29.4	39.3	61.0	75.2	82.1	93.2	100
	Redispersed and dried	1.6	7.1	10.4	21.1	29.0	38.8	61.7	72.2	84.4	94.9	100
	Dispersed again and dried	1.2	6.6	10.0	21.4	30.8	38.8	58.8	70.2	83.4	93.5	100
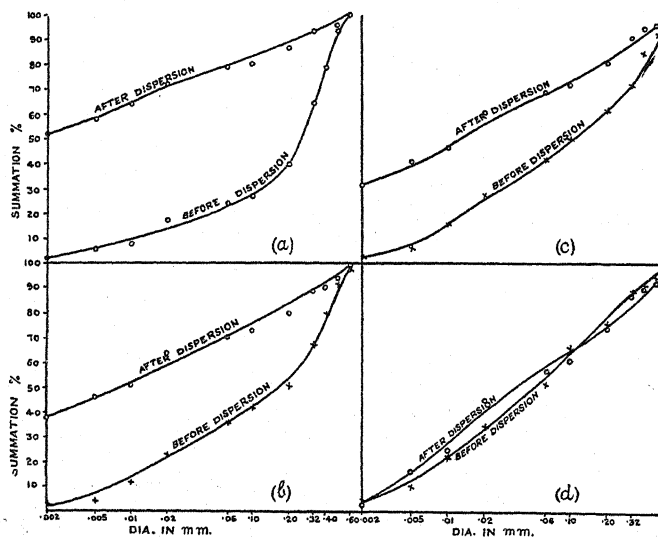
FIG. 1. EFFECT OF REMOVAL OF INCREASING AMOUNTS OF CLAY ON STATE OF AGGREGATION OF SOIL P. C. 13

(a) Sample dispersed and dried without removal of clay
(b) 50 per cent of total clay content removed
(c) 60 per cent of total clay content removed
(d) 90 per cent of total clay content removed

analysis. The mechanical analysis of the dried residual soils was made before and after dispersal by the sand method, and the results are given in figures 1, 2,

and 3 in the form of summation curves. It will be seen that on the removal of clay, the summation curves tend to become alike. This indicates less and less aggregation of the coarser fractions, and when the whole of the clay fraction has been removed the remaining fractions appear as in the completely dispersed soil.

The effect is exactly opposite when clay is gradually added to natural soils (figs. 4, 5, and 6). Clay for this purpose was separated from a dispersed clayey soil and was converted into hydrogen clay by treatment with dilute HCl and subsequent leaching with water to remove chloride ions. The clay was thus in a fully dispersed state and was stored without drying. Increasing amounts of this clay suspension were thoroughly mixed with the natural soils and dried. The
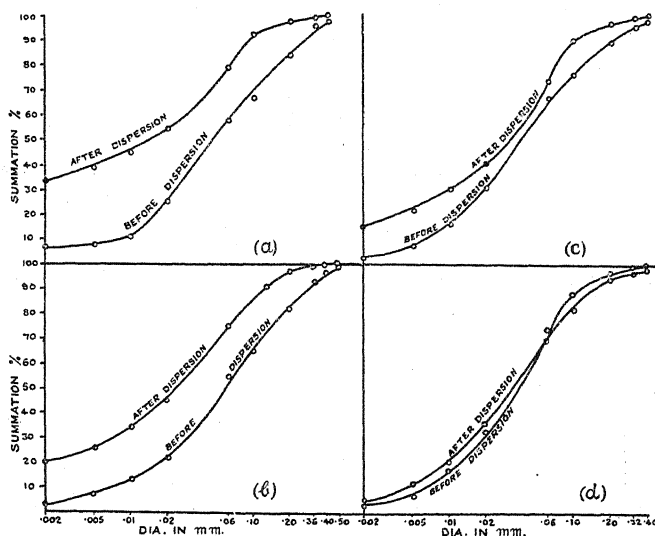
FIG. 2. EFFECT OF REMOVAL OF INCREASING AMOUNTS OF CLAY ON STATE OF AGGREGATION OF SOIL P. C. 6

(a) Sample dispersed and dried without removal of clay
(b) 50 per cent of total clay content removed
(c) 65 per cent of total clay content removed
(d) 90 per cent of total clay content removed

dried mixtures were powdered and analyzed as before. It might be mentioned that hydrogen clay once dried does not redisperse in water unless it is converted into sodium clay or given some violent mechanical treatment such as rubbing with a rubber pestle or shaking with sand. The dried samples therefore represented the state of aggregation which was water-stable.

These results confirm the conclusion previously reached that the state of aggregation of a soil, within limits, is a fundamental characteristic which is dependent on the ultimate mechanical composition of the soil.[4] The belief that

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[4] Puri, A. N., and Puri, B. R. 1939 Physical characteristics of soils: II. Expressing mechanical analysis and state of aggregation of soils by single values. *Soil Sci.* 47:77–81.
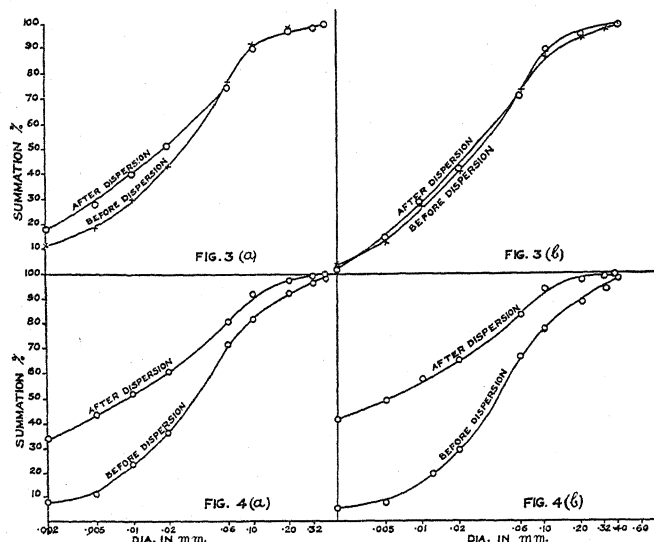
FIG. 3. EFFECT OF REMOVAL OF INCREASING AMOUNTS OF CLAY ON STATE OF
AGGREGATION OF FIELD LABORATORY SOIL

(a) Sample dispersed and dried without removal of clay
(b) 90 per cent of total clay content removed

FIG. 4. EFFECT OF ADDITION OF CLAY ON STATE OF AGGREGATION OF
FIELD LABORATORY SOIL

(a) 1.2 gm. of clay added to 10 gm. of soil
(b) 4.0 gm. of clay added to 10 gm. of soil
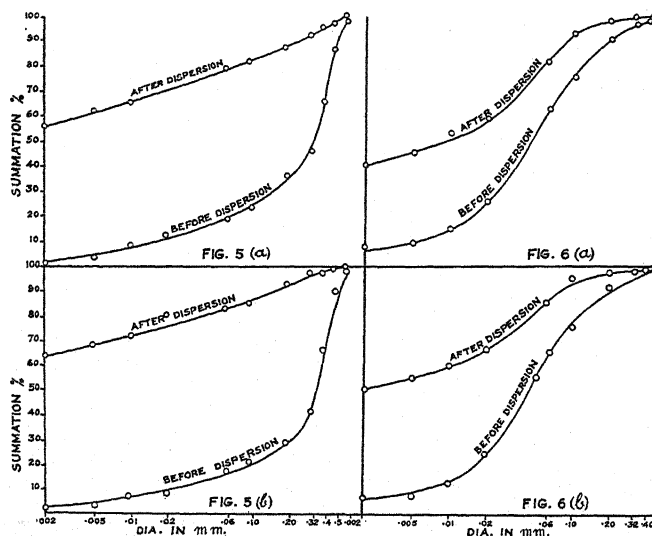
FIG. 5. EFFECT OF ADDITION OF CLAY ON STATE OF AGGREGATION OF SOIL P. C. 13

(a) 1.0 gm. of clay added to 10 gm. of soil
(b) 3.0 gm. of clay added to 10 gm. of soil

FIG. 6. EFFECT OF ADDITION OF CLAY ON STATE OF AGGREGATION OF SOIL P. C. 6

(a) 1.5 gm. of clay added to 10 gm. of soil
(b) 4.0 gm. of clay added to 10 gm. of soil

the quantity of water-stable crumbs in a soil may change from day to day is not well founded, and the mechanical analyses of soils without any preliminary treatment whatsoever are likely to yield useful results.

Another aspect of the state of aggregation of soils is the formation of aggregates on flocculation of a suspension. It is of interest to know whether these aggre-
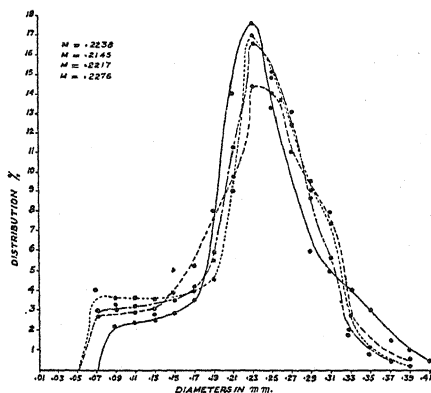
FIG. 7. SIZE DISTRIBUTION CURVES OF SOIL P. C. 13 FLOCCULATED WITH
CALCIUM CHLORIDE

M = mean diameter
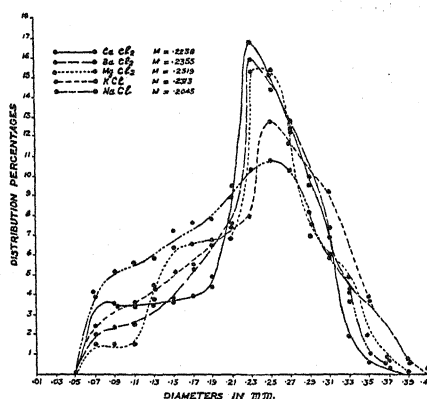
FIG. 8. SIZE DISTRIBUTION CURVES OF SOIL P. C. 13 FLOCCULATED WITH
DIFFERENT ELECTROLYTES

M = mean diameter

gates bear some relation to the crumb structure of the soil on drying or to its ultimate mechanical composition. For this purpose a completely dispersed H-soil suspension was flocculated by the addition of 10 cc. of N CaCl$_2$ to 2 gm. of soil in 100 cc. of water. The flocculated suspension was kept for 10 days to produce water-stable flocs, and then was analyzed in the Puri siltometer. The results of this analysis for soil P. C. 13 are given in figures 7 and 8 in the form of

distribution curves.   It will be seen that the curves are reproducible; in other words, the state of aggregation is not due to the chance coalescence of the particles but takes place in accordance with some definite relationship to the mechanical composition of the soil.   It is interesting to note that for soil P. C. 13 neither the shape of the distribution curve nor the mean diameter (M) differs very much when different flocculants are used, though sodium chloride appears to give a somewhat lower value for the mean diameter (fig. 8).   This is not surprising in view of the peculiar behavior of sodium soils.   Potassium, on the other hand, behaves like a divalent ion.

It was felt that the concentration of NaCl used for these experiments might not have been sufficient to bring about the same state of aggregation.   Raising the concentration of the solution from 0.1 to 0.2 N, however, did not materially affect the distribution curve.   These results show, therefore, that soils with sodium as the exchangeable base definitely give finer crumbs.

The flocculation experiment was repeated with a very fine alluvial soil containing about 85 per cent clay.   The mean diameters varied slightly, though the peak of the curve was found to lie at the same point in every case.   Since it is the clay fraction only which shows the flocculation effect to any marked degree, slight variations in the experimental conditions are likely to produce greater changes in a highly clayey soil.

CONCLUSIONS

Soil can be dispersed and dried repeatedly without effect on its crumb structure in the dry state.

Water-stable crumbs in a natural soil represent more or less permanent structures which are dependent on the ultimate mechanical composition of the soil and thus represent fundamental characteristics which are not likely to change from day to day.

Mechanical analysis of natural soils without any preliminary treatment, chemical or mechanical, is likely to prove of value in the light of this investigation.

BOOKS

Chemistry Made Easy. Third Edition. By CORNELIA T. SNELL AND FOSTER DEE SNELL. D. Van Nostrand Publishing Company, Inc., New York, 1943. In 4 volumes, containing 184, 232, 256, and 542 pages, respectively. Price, $7.95 for the set.

Volume one of this set of books deals with the theory of inorganic chemistry; volume two, with the elements and compounds in inorganic chemistry; volume three, with the aliphatic and aromatic compounds of organic chemistry; and volume four, with the chemicals of commerce. If chemistry can be made easy, these four volumes probably accomplish the purpose. The material is presented in an interesting and very concise manner, without troublesome detail. A list of questions is appended to each chapter. The fourth volume is of special interest in that it gives a brief description of most of the current industrial compounds and their composition, with general methods of production of most of them. The authors suggest the use of these four volumes for home reading on the part of persons who have not had the opportunity to become acquainted with this science in high school or college. The books appear to meet the requirements admirably for this purpose.

Tree Experts' Manual. By RICHARD R. FENSKA. A. T. De La Mare Company, Inc., New York, 1943. Pp. 192, illus, 48. Price, $4.50.

This book contains the answers to virtually every question one would ask about the growing of trees such as are planted in public parks, along city streets, and around residences. It includes chapters on selecting, transplanting, watering, fertilizing, pruning, spraying, dusting, bracing, and otherwise caring for trees. The book is exceptionally well illustrated, and the instructions are specific and to the point. The discussions on the root systems of trees and on methods of applying fertilizers are of special interest to those concerned with soils.

Food, War and the Future. By E. PARMALEE PRENTICE. Harper and Brothers, New York, 1944. Pp. 164, illus. 5. Price, $2.50.

The immediate concern of the author is not as indicated by the title of the book, but rather as to whether or not the agricultural colleges and experiment stations are doing the work assigned to them. His most serious criticism revolves around the word "purebred" as applied to the "registered" cattle of the United States. But he also raises a question concerning compensation from private interests which, if accepted by the teacher or research worker, may mean the loss of independence of position. The justification for the critical examination into what the agricultural college and station staffs are doing lies in the author's disturbance by the growth of populations as related to the land available for crop production, and the need for the development of a more adequate agricultural research program. The book merits careful reading on the part of those against whom the criticisms are leveled.

Soil Groups and Sub-Groups of South Africa. By C. R. VAN DER MERWE.
   The Government Printer, Pretoria, 1941. Pp. 316, plates 3, profiles 16.
   Price, 15s.

The topographic features of the area, together with the complexity of the
parent materials, have resulted in a great diversity of irregularly distributed
groups and types of soils in South Africa.   Thus podzolic, desertic, and lateritic
soils lie in close proximity to one another, with often very abrupt transitions
from one to the other.   Instances are given in which the climatic factors are
dominant and others in which the parent materials dominate the soil-forming
forces.   Profiles, in color, are shown for 16 of the most characteristic types of
soil.   The book presents a careful survey of South Africa on modern pedological
lines that should prove of great usefulness to persons in the area and should be
of much interest to soil scientists the world over.

<div align="right">THE EDITORS.</div>

SEMIMICRODETERMINATION OF THE EXCHANGE CAPACITY OF SOILS

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The phenomenon of base exchange in soils has been the subject of a large number of investigations. Methods designed for the determination of the exchange capacity have been reported (2, 3, 4, 5, 6, 7, 8). The macromethod used in this laboratory is a modification of the method proposed by Chapman and Kelley (2). The procedure is time-consuming, and furthermore requires an appreciable amount of soil and reagents. In order to obviate some of these difficulties, simplified methods of analysis have been suggested (1) but no method yet proposed has the advantages of a rapid semimicrotechnique. It was with this thought in mind that the method described in this paper has been developed.

METHOD

The proposed scheme of treatments and analysis is essentially a semimicro-modification of the Chapman and Kelley method (2). One-gram aliquots of soil, rather than the 25- or 50-gm. samples that have been generally used, are subjected to the specified treatments. The prolonged leaching process which involved the use of ammonium acetate is replaced with a series of short digestion periods, using a soil-ammonium acetate mixture, followed by centrifugation of the soil suspension. After removal of the excess ammonium acetate with methyl alcohol, the total ammonia fixed by the soil is liberated by a semimicro Kjeldahl distillation, and the ammonia is determined quantitatively with Nessler's reagent.

Reagents

The following reagents are used:

A. 1 N ammonium acetate (pH 7)
B. 10 per cent ammonium chloride
C. Saturated ammonium oxalate
D. Methyl alcohol (neutral)
E. 0.02 N sulfuric acid
F. Nessler's reagent (9)
G. Standardized ammonium chloride (50 p.p.m. NH_4)
H. Heavy magnesium oxide

Preparation of the soil and replacement of exchangeable bases

The homogeneity of the soil under consideration is obviously of particular importance in this type of determination. The significance of the results depends essentially upon the uniformity of the sample subjected to the treatments

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and analysis. It is therefore advisable that soil aggregates, subsequent to air-drying, be thoroughly disintegrated by grinding the soil with a hard rubber pestle (rock particles occasionally present should not be crushed under these conditions). The soil is subsequently passed through a 20-mesh screen and thoroughly mixed.

The moisture content of a 10-gm. sample of the prepared soil is determined by drying for 24 hours in an oven maintained at 100°C. Duplicate samples of air-dry soil, equivalent to exactly 1 gm. of oven-dry soil (soil dried under the conditions of the moisture determination), are used for the determination.

The aliquot of soil is transferred quantitatively into a 3 x 16 cm. centrifuge tube containing 25 cc. of ammonium acetate (A). The mixture is thoroughly agitated by hand in order to suspend temporarily as much of the soil as possible in the liquid phase. It is then digested for 25 minutes in a water bath maintained between 60 and 70°C. The contents are then centrifuged at a force of $10^4 \times$ gravity for 5 minutes. The supernatant liquid is decanted and a second 20-cc. portion of ammonium acetate added; the mixture is agitated, digested for 10 minutes, and centrifuged again. After three such treatments, the presence or absence of calcium is ascertained in the supernatant liquid. [Three cubic centimeters each of ammonium chloride (B) and ammonium oxalate (C) are added to 10 cc. of the solution. A white turbidity indicates the presence of calcium.] The digestion and centrifugation process described is continued until calcium (or other indicator ion) ceases to appear in the liquid phase. The soil is then digested for several minutes with a final 10 cc. of reagent and centrifuged. (Generally, the exchange complex is saturated with ammonia after three to five centrifugations have been completed.)

Ten cubic centimeters of methyl alcohol[2] (D) are added to the soil residue. The mixture is digested for 5 minutes in a water bath maintained at 45°C., followed by centrifugation. This procedure is continued until the color produced when 10 cc. each of the supernatant liquid and water and 2 cc. of Nessler's reagent (F) are mixed is equivalent to that produced with methyl alcohol and water. The soil is then digested with a final 10 cc. of methyl alcohol, centrifuged, and alcohol removed by decanting.

It is important that ammonium acetate and methyl alcohol be decanted from the soil residue as carefully as possible. The very small particles, of colloidal dimensions, which are most active in the exchange reactions, are also most easily dislodged from the soil mat formed by the centrifugation. Loss of such colloidal material may introduce appreciable error when the exchange capacity of a soil is of a low order of magnitude.

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[2] Results have shown that commercial methyl alcohol is generally acid or alkaline. In this case, the pH is adjusted to 7 with NH_4OH or HCl. Also, a slight coloration occurs with Nessler's reagent, indicating the presence of a trace of ammonia. The ammonia generally present, however, occurs in such trace quantities that contamination from this source is considered insignificant.

Determination of ammonia in the exchange complex

The ammonia held in the exchange complex by the soil is determined with the apparatus illustrated in figure 1. The soil is transferred quantitatively with a fine stream of water into the small Kjeldahl flask. The following are then added rapidly in succession; 1 gm. of granulated pumice, 5 gm. of magnesium oxide (H), and water to yield a total volume of about 150 cc. The receiving flask (a 100- or 200-cc. volumetric flask) contains 10 to 50 cc. of 0.02 N sulfuric acid (E) (the amount present should be sufficient to neutralize the anticipated total quantity of ammonia liberated by the soil). The microburner is adjusted to maintain rapid distillation of the contents (approximately 5 cc. of distillate per minute). About 50 to 100 cc. of distillate should be collected, depending upon the amount of ammonia present in the exchange complex of the soil. The
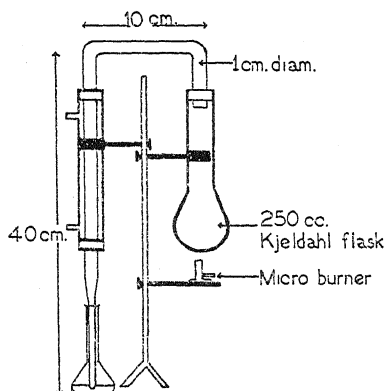
FIG. 1. APPARATUS FOR DETERMINING AMMONIA HELD IN THE EXCHANGE COMPLEX BY THE SOIL

fluid in the flask is subsequently diluted to volume. An aliquot containing 0.05 to 0.70 mgm. of ammonia is taken for analysis. The total ammonia is determined by Nessler's reagent (9). A 10-cc. aliquot of the ammonium chloride (G) is used as the standard of comparison. (The aliquot used in the analysis is diluted with water to 60–70 cc. in a 100-cc. volumetric flask. Five cubic centimeters of Nessler's reagent is added dropwise; meanwhile the contents of the container are shaken. Water is then added to the 100-cc. mark. The color developed in the aliquot taken from the distillate is compared with that produced with the ammonia solution in a Klett-Summerson photoelectric colorimeter.) A blank determination should also be made on the distillate obtained from a mixture of 100 cc. of water and 5 gm. of magnesium oxide.

RESULTS AND DISCUSSION

This semimicromethod was applied to a number of soils the exchange capacity of which had been measured by the macromethod. The results of both methods are presented in table 1. A comparison of the values shows that relatively ac-

TABLE 1

Comparison of exchange capacities of various soils as measured by the semimicromethod and the macromethod

LABORATORY SOIL NUMBER	SOIL TEXTURE	NH₃ PER 100 GM. OVEN-DRY SOIL			AVERAGE DEVIATION OF INDIVIDUAL DETERMINATION BY MICRO-METHOD VS. AVERAGE MACROVALUE	DIFFERENCE BETWEEN AVERAGE SEMI-MICRODETERMINATION AND AVERAGE VALUE BY MACRO-METHOD
		Semimicro method—individual determinations	Semimicro-method—average values	Macro-method—average values*		
		m.e.	*m.e.*	*m.e.*	*per cent*	*per cent*
102	Sand (coarse)	1.14	1.13	1.17	3.1	3.4
		1.13				
		1.09				
		1.17				
53	Sand	2.10	2.02	2.03	3.7	0.5
		1.91				
		2.09				
		1.99				
21D	Sand (fine)	3.68	3.68	3.66	1.6	0.6
		3.59				
		3.69				
		3.78				
29	Sandy loam	4.76	4.74	4.76	0.8	0.4
		4.70				
		4.70				
		4.79				
30	Fine sandy loam	4.95	4.90	4.95	1.6	1.0
		4.90				
		4.85				
78	Fine sandy loam	5.58	5.53	5.53	0.6	0.0
		5.49				
		5.52				
95	Loam	7.84	7.80	7.89	1.0	1.1
		7.75				
		7.82				
80	Sandy loam	12.43	12.42	12.44	0.2	0.1
		12.40				
101	Clay loam	17.30	17.23	17.35	0.6	0.7
		17.15				
90	Clay	18.72	18.71	18.70	0.1	0.1
		18.69				
100	Silty loam	20.28	20.28	20.28	0.1	0.0
		20.30				
		20.29				
36	Loam	24.65	24.69	24.75	0.1	0.2
		24.73				
77	Silty clay loam	25.92	25.86	26.00	0.5	0.5
		25.90				
		25.75				
38	Clay	30.00	30.00	30.00	0.0	0.0
		30.00				
40	Clay	33.80	33.89	33.97	0.2	0.2
		33.95				
		33.93				

* Individual average values of the exchange capacity determined by the macromethod are the results obtained from five or more determinations.

curate determinations of exchange capacities can be made on 1-gm. samples of soils over a range of values from 1 to 34 m.e. of ammonia per 100 gm. of soil. It seems probable that soils with exchange capacities greater than those mentioned here could be satisfactorily measured by this method. Soils with a value less than 1.1 have not been encountered in this laboratory. From a consideration of the types of soil studied (table 1), it would seem that the physical texture of the soil had little effect upon the accuracy of the determination.

It is of interest to note that on the basis of the limited number of analyses presented, the percentage deviation between the average macrovalues and semimicrovalues tends to become appreciable with an exchange capacity of 3 m.e. or less (fig. 2). From a practical viewpoint, however, an error of 5 per cent or less is relatively unimportant for a soil the exchange capacity of which is of the order of 1 or 2 m.e. per 100 gm. of soil.
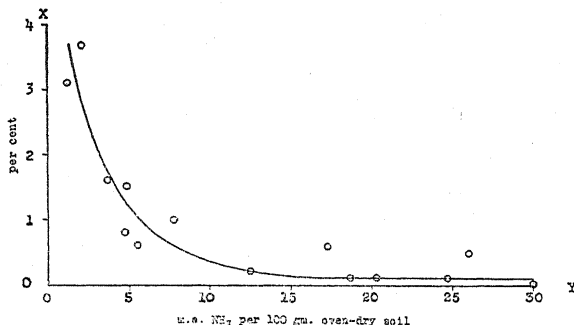
FIG. 2. PERCENTAGE DEVIATION BETWEEN AVERAGE MACROVALUES AND SEMIMICROVALUES FOR EXCHANGE CAPACITIES OF SOILS

X, average deviation of individual exchange-capacity measurements by the semimicromethod from the average exchange capacity determined by the macromethod.

Y, average exchange capacity determined by the macromethod.

From a comparison of the results obtained with the semimicromethod and the procedure of Chapman and Kelley, the author feels justified in proposing the method outlined as a rapid means of determining the exchange capacities of soils.

SUMMARY

A rapid semimicromethod has been described for the determination of the exchange capacity of soil. Comparisons have been made between results obtained by this method and those of the standard macromethod. A saving of time and reagents necessary to the measurements, without sacrifice of accuracy, has been demonstrated.

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THE FIXATION OF ADDED BORON BY DUNKIRK FINE SANDY LOAM[1]

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Previous work at this laboratory (18) has shown that the level of boron supply may have considerable effect on mineral accumulation by plants. The effects reported were of sufficient magnitude to suggest that boron supply may definitely affect the nutritive value of crop plants. In this respect the soil chemistry of boron may be of greater importance than was formerly thought. The present study was undertaken to obtain more information on the mechanism of boron fixation, in order to draw more generally applicable conclusions regarding the fate of boron amendments.

A number of workers have found that overliming certain soils results in plant deficiency symptoms, but that in many cases the deleterious effect of large lime applications does not occur where boron applications have been made (3, 12, 14, 26). This "overliming injury" is usually accompanied by a decreased boron content in the plants (2, 13, 26). One explanation of this phenomenon is that the deficiency symptoms noted are due to a physiological unbalance in boron and calcium supplies available to the plants (5, 8, 9, 10, 21), and that the soil boron is not actually "fixed" in a form unavailable to plants (5).

Other investigators have found, however, that when some soils are overlimed, a greater amount of a boron application is retained by the soil than by the same soils in an unlimed state (11, 12, 20). It has been suggested that this type of fixation is due to the stimulation of the soil microflora by liming, with attendant competition between crop plants and soil microorganisms for the available boron (2, 7, 15, 25). Studies with sterile soils do not support this contention, as it has been shown (11) that boron may be fixed in a period as short as 1 hour. The conclusion reached was that when podzolized soils are limed, boron reacts with or is adsorbed by the soil organic matter. Still other workers have found boron fixation to be a relatively slow process (6, 13) probably due to some type of chemical reaction (6, 26). In a previous paper (17), it was suggested that boron may enter into aluminum-silicate crystal lattices, possibly in substitution for aluminum.

MATERIALS AND METHODS

The soil used was Dunkirk fine sandy loam, collected from the surface 6 inches of an experimental field near Ithaca, New York. After being dried and mixed,

a portion of the soil was used without further treatment, and the remainder extracted with 0.05 N HCl in the following manner. Ten liters of 0.05 N HCl were added to 25 pounds of soil in a 5-gallon glazed crock. The mixture was stirred, allowed to settle for 24 hours, and the supernatant liquid siphoned off. More acid was then added, and the treatment repeated seven times. Six similar extractions were then made with distilled water, until only a faint chloride test was obtained. Sedimentation was excellent for both acid treatments and washings. The soil was then dried, rolled, and mixed. Hereafter this material will be referred to as "acid-treated soil" and the original sample as "normal soil."

A series of incubation experiments were carried out in 4-inch soft-glass tumblers. To the tumblers were added 125-gm. samples of soil and 40 ml. (slightly over saturation) of aqueous borax solution to give the following boron concentrations, in parts per million of soil on a dry-weight basis: 0.00, 0.06, 0.28, 1.42, 4.26, and 11.36. These concentrations are analogous to field applications of borax ($Na_2B_4O_7 \cdot 10H_2O$) at the rates of 0, 1, 5, 25, 75, and 200 pounds per acre. The samples were then dried in forced-draft ovens at varying temperatures. After drying, the samples were saturated with 40 ml. of distilled water and dried again. In certain series as many as 24 such drying cycles were carried out. Since crust formation tended to occur, each sample was removed after every third drying cycle, rolled, mixed, and replaced in the tumbler.

After varying treatments and incubations the samples were removed from the tumblers, rolled, and mixed, and extractable boron was determined on duplicate samples of soil from each tumbler. The refluxing technique of Berger and Truog (1) was used in making the extractions (20 gm. soil boiled 5 minutes with 40 ml. water). Boron extracted by this method has been shown (1, 4) to be a fairly reliable measure of available boron. Water-clear extracts were obtained by centrifuging the soil-water mixtures for 45 minutes at 1700 r.p.m., 250-ml. bottles being used in an International Equipment Company laboratory centrifuge, size 1, type SB (13 cm. from axis to trunnion carrier pivot). Boron was determined on the extracts by the method of Naftel (16), aliquots of such size being used that the quantity of boron actually determined varied from 0.5 to 5.0γ.

Preliminary studies as to the suitability of the Naftel (tumeric-oxalic) method for this type of study are reported in tables 1 and 2. The extracts used were from soils with treatments analogous to those of the samples subsequently discussed. The data in table 1 indicate that neither ignition [gas hot plate after drying with $Ca(OH)_2$] nor size of aliquot had any appreciable effect on the determination of boron by this method. The various aliquot sizes represent indirect recovery tests, as well as showing the absence of interfering substances. The results of further recovery trials are shown in table 2. In addition to the excellent recoveries of added boron when clear extracts were used, two other points are worthy of mention. For the extracts containing traces of suspended clay, the recoveries of added boron are strikingly low. Where no boron was added to the extracts, however, the boron determinations on the turbid extracts are definitely higher than those on the clear ones. These two phenomena

are consistent with other data that will be discussed in connection with the fixation studies reported in a later section.

TABLE 1

Effects of aliquot size and of ignition on determination of boron by the Naftel method

SAMPLE NUMBER	ALIQUOT OF SOIL EXTRACT	BORON PER ALIQUOT	BORON PER GRAM OF SOIL	IGNITION
	ml.	γ	γ	
80a	0.5	1.00	4.00	+
80b	0.5	1.05	4.20	−
80c	1.0	2.15	4.30	+
80d	1.0	2.10	4.20	−
80e	2.0	4.25	4.25	+
80f	2.0	4.10	4.10	−
81a	2.0	0.15	0.15	−
81b	5.0	0.38	0.15	−
81c	10.0	0.70	0.14	−

TABLE 2

Effects of suspended material in aqueous extracts of Dunkirk fine sandy loam on determination and recovery of boron

SAMPLE NUMBER	CENTRIFUGED UNTIL	BORON DETERMINED	BORON ADDED	TOTAL BORON PRESENT	TOTAL BORON FOUND	RECOVERY
		γ	γ	γ	γ	*per cent*
73a	Clear	2.60	2.00	4.60	4.70	102
73b	Slightly turbid	2.80	2.00	4.80	3.80	79
76a	Clear	2.70	2.00	4.70	4.70	100
76b	Turbid	3.00	2.00	5.00	3.60	72
2a	Clear	0.38	1.00	1.38	1.45	105
3a	Clear	0.70	1.00	1.70	1.63	96

EXPERIMENTAL

The effect of increasing the number of drying cycles on the fixation of boron by the acid-treated soil is shown in figure 1. Series of samples were caried at two levels of boron through 0, 2, 5, 11, and 24 drying cycles. The boron "fixed" was calculated by subtracting the boron extracted, from the boron originally present (extractable native boron plus added boron). The extractable native soil boron was 0.09 p.p.m. for the acid-treated soil, 0.05 p.p.m. for the normal soil. The boron concentrations are presented in all cases as parts per million of soil on a dry-weight basis. The value for 0 number drying cycles was obtained by adding the correct amount of boron in the extraction water to the soil sample

just before refluxing. The sample was then boiled, centrifuged, and carried through the regular procedure. These values are indicative of the completeness with which boron, known to be in soluble form, is removed by this extraction procedure. The effect of drying on boron fixation was marked. The similarity of the two curves indicates the same type of response at different boron levels. The curves also indicate, however, a more nearly complete fixation of the low level of boron addition than of the high level, for a given number of drying cycles. The effects of drying and of total time of contact are not differentiated in this experiment.

Results showing the effect of temperature of drying on boron fixation are presented in figure 2. Each sample contained 11.35 p.p.m. of extractable boron at the beginning of the experiment, and the drying temperatures used were 26°,
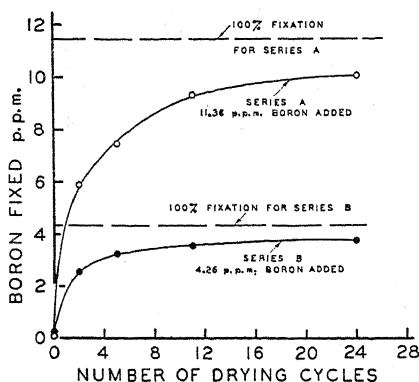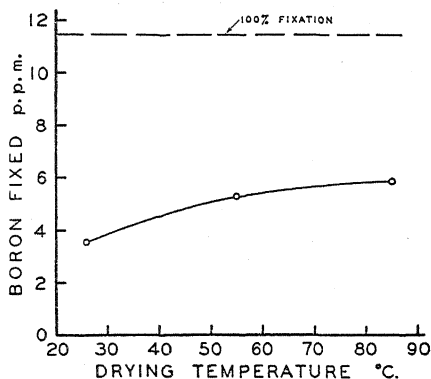
FIG. 1. EFFECT OF ALTERNATE WETTING AND DRYING AT 85°C. ON THE FIXATION OF BORON BY ACID-LEACHED DUNKIRK FINE SANDY LOAM

FIG. 2. EFFECT OF DRYING TEMPERATURE ON FIXATION OF BORON BY ACID-LEACHED DUNKIRK FINE SANDY LOAM

Samples carried through two cycles of alternate wetting and drying

55°, and 85° C. The samples were carried through only two drying cycles, which probably accounts for the fixation of only 30 to 50 per cent of the boron present. Though the effect of temperature is of considerable importance in postulating a mechanism of boron fixation, it is apparent that temperature of drying is not so critical a factor in determining boron fixation as is the number of drying cycles.

Since boron deficiency has been associated with liming of soils, a set of samples was prepared to determine boron fixation at several lime levels. Each sample contained 11.36 p.p.m. of extractable boron at the beginning of the experiment, and the lime used was a mixture of $CaCO_3$ and $MgCO_3$ (25 per cent of the neutralizing power from the latter) at rates equivalent to 0, 1, 4, and 12.5 tons of $CaCO_3$ per acre. The lime and boron were added together, and the samples carried through five drying cycles. The results of this study, reported in figure 3, are not in accord with the results and assumptions made on the basis of field

studies by most other investigators, since the effect of increasing the lime level was to *decrease* the amount of boron fixed. It is also of considerable interest to note that this decrease in fixation is more nearly a straight line function of increasing soil pH than of increasing lime applications. A similar relationship in field soils has been noted in Hawaii (24), where a highly significant relationship was found between increasing amounts of available boron and increasing soil pH values.

A more complete study was therefore initiated to determine, at a wide range of boron levels, the effect of varying sequences and combinations of lime and boron additions on boron fixation. In figure 4 are presented the results of two series of samples in which the variable was the presence or absence of lime in the soil before boron additions. In series B, dolomite (equivalent to 12.5 tons of lime per acre) was mixed with the samples, and the soils were carried through 13 drying cycles. Samples of series A were carried through the same 13 drying cycles, but without the addition of lime. Boron was then added in varying amounts to the samples of both series, and all soils were carried through another 11 drying cycles. The previously noted effect of lime in decreasing boron fixation is even more strikingly evident here [fig. 4 (upper)], where the lime treatment was present before the boron was added, than in figure 3, where the lime and boron were added simultaneously. [The two lowest levels of boron addition are omitted in figure 4 (upper).] In curve A, fixation is apparently a straight-line function of the amount of boron present, whereas in curve B there is a slight tendency for the curve to fall off in the higher concentrations. This effect is better illustrated in figure 4 (lower), where for each original concentration of extractable boron, the percentage of the original concentration which was fixed is recorded. Where lime was not present (curve A), approximately 80 per cent of the boron present in each sample was fixed, regardless of the magnitude of this original concentration. The one exception is the sample in series A to which no boron was added, in which case only slightly over 40 per cent of the originally extractable boron was fixed during the 24 drying cycles. In curve B of this figure, representing the samples in which lime applications preceded the boron additions, an initial *increase* and a subsequent decrease in *percentage* fixation is very clear. An increase in percentage fixation of boron by soils, with increasing boron concentrations has also been shown previously (24).

The rather unusual effect of increased percentage fixation with increased concentration is even better illustrated by the data in table 3. In these series, whether lime was worked into the soil by wetting and drying and then boron was added, or whether the boron was allowed to react with the soil before the lime was added, the effects of lime and dolomite were exactly the same, and the effect of initial lime applications to decrease boron fixation at the higher boron levels is again to be noted.

Where lime was added after the boron (treatment C), the increase in percentage fixation with increased concentration is more gradual than where boron was present for 11 drying cycles and with no subsequent lime addition (curve A, fig. 4, lower). The increase in percentage fixation from 80 per cent to 86 per cent
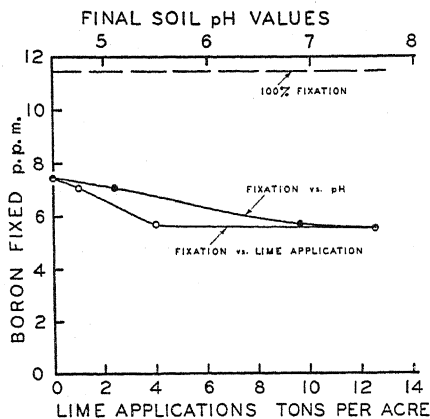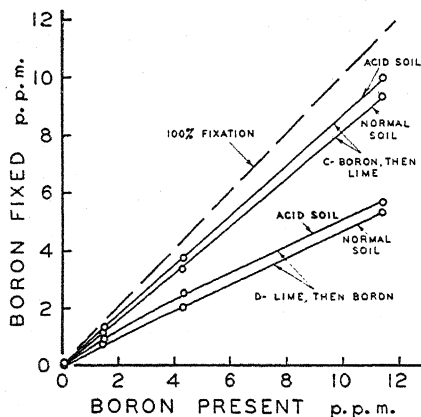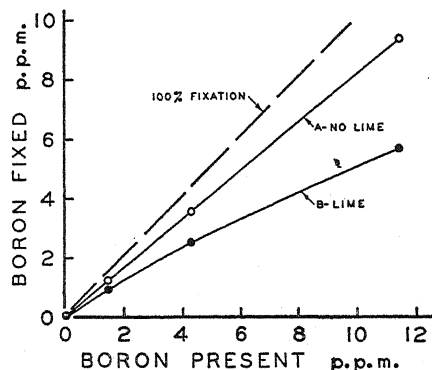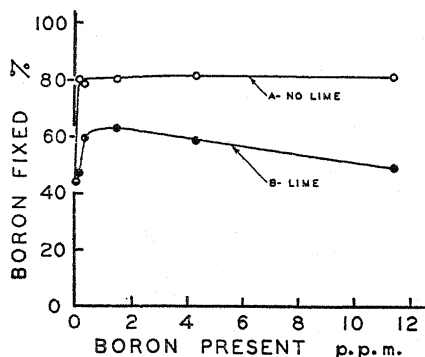
FIG. 3. FIXATION OF BORON BY ACID-LEACHED DUNKIRK FINE SANDY LOAM IN RELATION
TO APPLICATIONS OF LIME, AND TO ACCOMPANYING CHANGES IN SOIL pH

Lime and boron added simultaneously at the beginning of the experiment; 5 drying cycles,
85°C. drying temperature

FIG. 4. EFFECT OF BORON ADDITIONS ON THE QUANTITATIVE (ABOVE) AND PERCENTAGE
(BELOW) FIXATION OF BORON BY ACID-LEACHED DUNKIRK FINE SANDY LOAM,
WITH AND WITHOUT LIMING

24 drying cycles, 85°C. drying temperature. Lime present (curve B) for all 24 drying
cycles. Boron additions present (curves A and B) for the last 11 drying cycles

FIG. 5. EFFECT OF ORDER OF ADDITION OF BORON AND LIME ON FIXATION OF BORON BY
ACID-LEACHED AND NORMAL DUNKIRK FINE SANDY LOAM

C, boron additions present for all 24 drying cycles, lime present during last 11 drying
cycles. D, lime additions present for all 24 drying cycles, boron additions present for
last 11 drying cycles.

(fig. 4, table 3) may be accounted for either by delayed lime additions or by the increased number of drying cycles during which boron was present in the soil. The increase is of such magnitude that it could readily be accounted for by the increased number of drying cycles.

This point is also subject to examination in the results presented in table 4. The comparison of two series of treatments for both acid-treated and normal soil shows a definite, but rather small, increase in fixing power due to the acid treatment. In all samples the boron additions were present for all 24 drying cycles, and there is no evidence of any increase in fixing power for the series where limed additions were made after the thirteenth drying cycle. The increase in percentage fixation with increasing boron concentrations for the normal soil

TABLE 3

Effect of lime and dolomite on fixation of boron by acid-leached Dunkirk fine sandy loam
24 drying cycles, 85°C. drying temperature

BORON PRESENT*	BORON FIXED		AVERAGE BORON FIXED	TREATMENT
	Lime	Dolomite†		
p.p.m.	*p.p.m.*	*p.p.m.*	*per cent*	
0.09	0.02	0.02	22.2	C. Boron additions present for all
0.15	0.05	0.06	36.7	24 drying cycles; lime present
0.37	0.28	0.27	74.3	during last 11 drying cycles
1.51	1.29	1.33	86.7	
4.35	3.78	3.75	86.6	
11.45	9.85	9.95	86.5	
0.09	0.02	0.04	33.3	D. Lime additions present for all 24
0.15	0.07	0.07	46.7	drying cycles; boron additions
0.37	0.17	0.21	51.4	present for last 11 drying cycles
1.51	0.93	0.95	62.3	
4.35	2.60	2.55	59.2	
11.45	5.75	5.65	49.8	

* Native extractable (0.09 p.p.m.) plus added boron.
† From c.p. carbonates. One-fourth neutralizing power contributed by $MgCO_3$.

series is far more striking than that for the acid-leached soil, since at the lower concentrations there was either no fixation or an actual release (presented as negative fixation) of soil boron. The trends, however, are of the same general pattern as for the acid soil series.

The normal and acid-treated soils are further compared in figure 5. The data show that whether the treatment was one which produced high or low fixation, the two soils were slightly different and stood in the same relation to one another, throughout the range of boron concentrations used.

Although this study was designed to obtain information as to the fate of boron additions, a few data were obtained dealing with the fixation of native extractable soil boron as influenced by the treatments used. These data are presented to-

TABLE 4

Effect of boron additions on boron fixation in acid-leached and normal Dunkirk fine sandy loam where no lime was applied, or where lime was applied after the thirteenth drying cycle

Boron additions were present in all cases for all 24 drying cycles; 85° C. drying temperature

BORON PRESENT*	BORON FIXED				SOIL
	Lime		No lime		
p.p.m.	p.p.m.	per cent	p.p.m.	per cent	
0.09	0.02	22.2	0.05	55.5	Acid-leached
0.15	0.06	36.7	0.07	46.7	
0.37	0.27	74.3	0.27	73.0	
1.51	1.33	86.7	1.29	85.4	
4.35	3.75	86.6	3.75	86.2	
11.45	9.95	86.5	10.05	87.8	
0.05	−0.04	−80.0	−0.03	−60.0	Normal
0.11	0.00	0.0	−0.02	−18.2	
0.33	0.18	54.5	0.14	42.4	
1.47	1.19	80.9	1.17	79.6	
4.31	3.38	78.4	3.34	77.5	
11.41	9.31	81.6	9.21	80.7	

* Native extractable (0.09 p.p.m. or 0.05 p.p.m.) plus added boron.

TABLE 5

Effects of liming and of drying on fixation and release of native soil boron by acid-leached and normal Dunkirk fine sandy loam

SAMPLE NUMBER	EXTRACTABLE BORON		NUMBER OF DRYING CYCLES	SOIL	TREATMENT
	Original	Final			
	p.p.m.	p.p.m.			
1	0.09	0.07	24	Acid	12½ T. lime for last 11 dryings
7	0.09	0.07	24	Acid	12½ T. dolomite for last 11 dryings
13	0.09	0.07	24	Acid	12½ T. lime for all 24 dryings
19	0.09	0.05	24	Acid	12½ T. dolomite for all 24 dryings
25	0.09	0.04	24	Acid	None
49	0.09	0.05	24	Acid	None
31	0.05	0.09	24	Normal	12½ T. dolomite for last 11 dryings
37	0.05	0.09	24	Normal	12½ T. dolomite for all 24 dryings
43	0.05	0.08	24	Normal	None
64	0.09	0.06	5	Acid	None
65	0.09	0.07	5	Acid	1 T. dolomite for all 5 dryings
66	0.09	0.05	5	Acid	4 T. dolomite for all 5 dryings
67	0.09	0.03	5	Acid	12½ T. dolomite for all 5 dryings

gether in table 5. In the acid-leached soils, regardless of treatment or number of drying cycles, boron fixation occurred but was not of great magnitude. For the acid soil samples dried five times and treated with increasing amounts of lime, there is a tendency for increased fixation with increased lime level. This is the reverse of the analogous series with boron additions (fig. 3) and of all the other treatments involving the effect of lime on the fixation of added boron. There was an actual release of boron in the normal soil samples, regardless of treatment.

The question immediately arises as to whether phenomena of an entirely different nature are taking place in the normal and acid-leached soils at the lowest levels of boron concentration. This question is answered in part by the cuvres
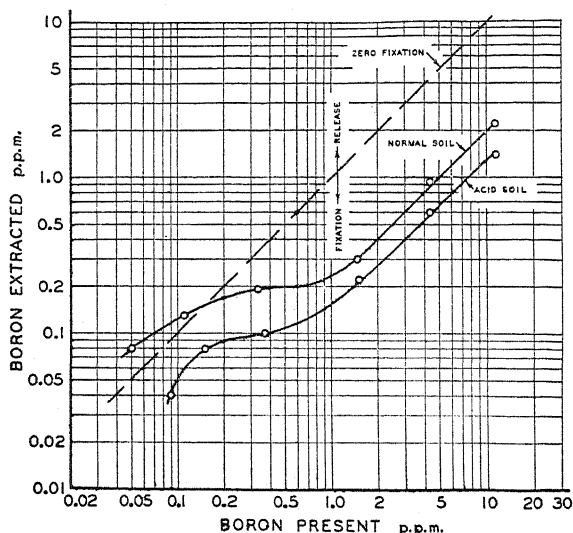
FIG. 6. — FFECT OF BORON ADDITIONS ON AMOUNT OF BORON EXTRACTED FROM ACID-LEACHED AND NORMAL DUNKIRK FINE SANDY LOAM

Boron additions present for all 24 drying cycles; no lime applications; 85°C. drying temperature

presented in figure 6. The boron additions were present in all cases for all 24 drying cycles. No lime applications were made. The curves for both soils are of the same general shape, and the part of the normal soil curve showing release is continuous with the part of the curve representing fixation. The apparent equilibrium shift toward smaller percentage fixation at the lower boron concentrations is also more apparent for the normal than for the acid soil.

DISCUSSION

The wetting and drying treatments used were particularly effective in reducing the amount of hot-water-extractable boron in soils treated with borax. Amounts of boron extracted in this way have been found to correlate very well (1, 4) with quantities of boron available for plant growth. Since the effect of lime treatment was, with one exception, to decrease strikingly the amount of boron fixa-

tion, considerable support is given to the school of thought (5, 8, 9, 10, 21) which holds that such overliming injury as can be remedied by boron additions is a physiological effect, due to a lack of balance in boron and calcium supplies to the plant.

It is also significant to note, in connection with the liming effects reported here, that on soils subject to overliming injury, a borax application of 10 or 20 pounds per acre will often remain effective for 2 or sometimes 3 years.

Whether or not overliming injury and boron fixation are shown to be non-coincident effects, it will also be necessary from the standpoint of agronomic practice to know whether fixed boron eventually becomes available for plant growth (as in the case of fixed potassium) and the extent of the time intervals involved.

Another point which seems to merit further investigation by simultaneous soil and plant studies is the effect of drying on both boron deficiency in plants and boron fixation in soils. Boron fixation may be shown to be of particular agronomic significance in connection with reports (9, 19, 22, 23) that boron-deficiency symptoms are aggravated by periods of dry weather, whereas during a wet season, crops on the same field will show no evidences of boron deficiency. A discrepancy exists, however, since in this study drying was shown to fix large amounts of added boron, but not of native extractable boron. Although the extractable boron of the acid-treated soils decreased with drying, the extractable boron of the normal soils remained the same, or increased, after a number of drying cycles.

The results reported here tend to support the previously suggested (17) mechanism of boron fixation as an actual entrance of boron into the clay crystal lattice surfaces. The increase in boron fixation, with increased temperatures up to 85° C., and the large difference in fixation depending on whether the lime was added before or after the boron, as well as the effect of increasing the number of drying cycles, are not consistent with the concept of fixation by microbiological activity. Adsorption of boron on organic or clay colloids would be expected to conform to the general pattern of adsorption reactions. In these studies, the opposite trend was evident, that is, at the lower levels, as boron concentration increased, percentage fixation also increased. Chemical precipitation as a possible mechanism of boron fixation is not eliminated by the data reported here. The importance of the sequence of boron and lime additions in affecting fixation is, however, rather difficult to explain on the basis of such a reaction. The effects of temperature, number of times of drying, and increase in percentage fixation with increase in boron present, are all consistent with an actual reaction of boron with crystal lattice surfaces. The apparent steric effect of lime additions preceding the boron applications is also most readily explained on the basis of such a mechanism.

It will be recalled that in presenting the results of extraction studies (table 2), solutions containing suspended clay and dried as part of the analytical procedure, gave high values if no boron was added, and low recoveries where boron was added to these extracts. It seems quite consistent that in the incubation studies

the effect of drying the normal soil was to increase fixation of added boron and release of native soil boron where no boron was added. It would, therefore, seem likely that the low and high recoveries reported in table 2 (also extracts of normal soil) were evidences of boron fixation and release by the clay suspended in the soil extracts.

SUMMARY

Soil incubation experiments were carried out to obtain further information as to the nature of the process of fixation of boron additions. The soils used were normal and acid-leached Dunkirk fine sandy loam.

The variables studied in relation to boron fixation were temperature of drying the soil, number of drying cycles, amount of lime, type of liming material, amount of boron added, and various sequences of boron and lime additions.

Fixation of added boron varied from none to almost complete fixation with increase in number of drying cycles. Increase in temperature of drying from 26 to 85° C. almost doubled the amount of boron fixation. Lime, added with boron, or before boron additions, strikingly decreased the fixation capacity of the soils for boron. At the lower levels of boron supply, percentage fixation increased as boron concentrations increased.

The data presented are discussed in the light of the work of other investigators. It is concluded that the data obtained tend to support the mechanism of boron fixation by entrance into the clay crystal lattice, more than fixation by chemical precipitation, adsorption by clay or organic matter, or microbiological fixation.

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NOXIOUS NITROGEN IN LEAVES, CROWNS, AND BEETS OF SUGAR BEET PLANTS GROWN WITH VARIOUS FERTILIZERS

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The basic or noxious nitrogen of beets consists primarily of lysine, glutamine, arginine, asparagine, and betaine. It is of special importance for the following reasons: (*a*) It tends to concentrate in the molasses, interfering with the crystallization of the sugars and unsuiting the molasses for human consumption. (*b*) Milk cows fed on products containing large quantities of betaine produce milk that develops off-flavors (7, 8). The betaine may be changed to amines that directly flavor the milk, or it may be transformed during normal metabolism to choline, which may increase the lecithin content of the milk and butter. Lecithin on hydrolysis may give rise to fishy-flavored products. (*c*) In siloed beet-products, toxic substances that do not have the characteristic of botulism toxin occasionally develop. Theoretically these toxic substances may result from the transformation of betaine into muscarine-like compounds. Where the beet products are fed directly, however, they have a special nutritive value. It is now known, for instance, that animals receiving an insufficient quantity of choline may develop fatty livers and fail to make a normal gain in body weight. In the rat, choline is necessary for normal lactation, and paralytic conditions similar to those in B_1 avitaminosis may develop if the supply is inadequate. In chickens and turkeys perosis may develop in the absence of choline. Choline, which can be synthesized from betaine, apparently plays three important roles in animal metabolism: (*a*) It functions in the formation of phospholipids, which are vital in fat and phosphorus metabolism; (*b*) it is essential for the production of acetylcholine, which in turn is essential for normal nerve functions; (*c*) it supplies labile methyl groups, which are required. For these reasons it is important that we know the quantity and distribution of noxious nitrogen in the sugar beet.

MATERIALS AND METHODS

Production of beets

The beet plants used in this investigation were grown on a well-drained Millville loam soil with a sandy loam subsoil. The permanent water table is about 100 feet below the surface. The chemical and physical composition of the soil is similar to that of the adjoining Greenville Experimental Farm soil, which is described elsewhere (4). The soil is rich in potassium and phosphorus but comparatively low in nitrogen. Because of a high content of calcium and magnesium carbonates, it is slightly basic.

The soil lies in a productive area and can receive an optimum quantity of water; yet because of previous methods of farming without manure or fertilizers, the yields have been materially reduced.

The beet plants were grown on 1/20-acre plots. The commercial fertilizers were applied broadcast to each plot in the spring before the seedbed was prepared and were harrowed into the soil. The manure was applied each fall just before plowing.

The fertilizer treatments of the plots were as follows:

Plot 29, check V_2, no fertilizer, located between plots supplied with potassium and with nitrogen and potassium.

Plot 36, check V_1, no fertilizer, located between two manured plots.

Plot 37, manure, 10 tons per acre (1 ton of manure contained approximately 728 pounds dry matter, 3 pounds phosphorus, and 16 pounds nitrogen).

Plot 38, manure 10 tons, raw rock phosphate 1000 pounds, and sulfur 250 pounds per acre (the composition of the manure was the same as that used on plot 37).

Plot 39, gypsum, 330 pounds per acre.

Plot 40, nitrogen, 240 pounds ammonium sulfate, 20 per cent N, per acre.

Plot 41, phosphorus, 350 pounds triple superphosphate, 45 per cent P_2O_5, per acre.

Plot 42, potassium, 167 pounds potassium chloride, 61 per cent K_2O, per acre.

Duplicate samples were collected at random and consisted of 17 to 24 beet plants each, depending on the number in the plot. The plants were thoroughly washed and prepared for analysis as follows:

Leaves. The leaves were removed, with just sufficient beet adhering to hold them together. The leaves were weighed. The adhering crown was cut in two across the center, and alternate triangular sections of leaves from circumference to center were removed. In this way relative amounts of leaves of all ages were obtained. Comparable portions were ground and dry matter was determined; the remainder was dried, ground, and preserved for analysis.

Beets and crowns. The beets were weighed and the crowns removed at the lowest leaf scars. The crowns were divided into three sections: C_1, nearest the leaves; C_3, nearest the beet; and C_2, between C_1 and C_3, as illustrated in figure 1. Alternate triangular sections were removed from each half of each beet. Dry matter was determined on portions of beets and crowns. Other portions were dried, ground, and preserved for analysis.

Methods of analysis

The noxious nitrogen in the beets, crowns, and leaves was determined (11, pp. 200–201) by digesting 32.52 gm. of finely ground product in sugar flasks containing 165–170 ml. of distilled water for 15 minutes at 90°C. To this was added 13 ml. of a copper hydroxide suspension[1] and 3.2 ml. of a 20 per cent solution of aluminum sulfate. The mixture was made up to 200 ml. with distilled water and then heated on a water bath for 30 minutes. On cooling it was made up to 201 ml. and filtered. This is referred to, as "Filtrate F."

[1] The suspension was prepared as follows: 200 gm. $CuSO_4.5H_2O$ plus 20 ml. of glycerine was dissolved in 10 l. of distilled water, made slightly alkaline with sodium hydroxide, thoroughly shaken, then let stand until the precipitate settled out; the supernatant liquid was decanted, and the precipitate washed twice by decantation. filtered off and suspended in 2 l. of distilled water.

To 50 ml. of filtrate F was added 1.5 ml. of concentrated sulfuric acid. This was evaporated on a water bath to a thick syrup and digested with sulfuric acid, and its nitrogen was determined by the Kjeldahl method. The resulting nitrogen is referred to as "A." To 75-ml. portion of filtrate F was added 1.5 ml. of concentrated sulfuric acid. This was digested for 2 hours at constant volume. Magnesium oxide was added, and the ammonia was distilled into 0.1 N acid. This represents the ammonia and amide nitrogen, and is designated as "B." Then $A - B$ = noxious nitrogen.
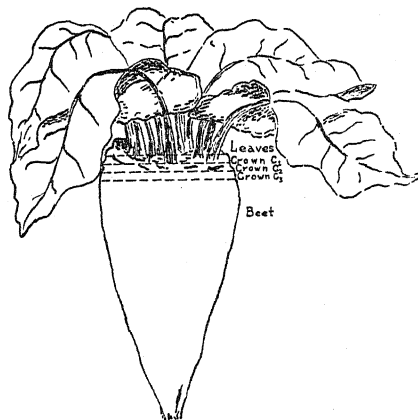
FIG. 1. DIVISION OF SUGAR BEET PLANT INTO LEAVES, CROWNS, AND BEET

RESULTS

Table 1 presents the average percentages of noxious nitrogen in leaves, crowns, and beets of sugar beet plants receiving various treatments. Table 2 gives the analysis of variance for these data. Since the plot treatments were replicated only once, no valid estimate of error exists for testing the significance of the treatment effects. Since two samples were selected at random from each plot, a valid estimate of error is available for testing the significance of the difference in noxious nitrogen from the several locations in the plant. The average difference in nitrogen content between plant sections is highly significant as shown by the F value of 0.5924/0.0037 which greatly exceeds the tabular value of F at $P = .01$.

The average percentage of noxious nitrogen was greatest in the leaves, followed by the crowns in order of nearness to leaves. The crown next to the beet was lowest in noxious nitrogen, but even this was higher than the beet.

Although no error is available for testing effect of treatment, it seemed worth while to isolate the mean squares for the various treatment effects. The average mean square for between treatments was 0.0532 with 7 degrees of freedom. Since there were two check plots and six fertilizer treatments, it is possible to break down the 7 treatment degrees of freedom into 1 for between checks, 5 for between the various fertilizers, and 1 which measures the effect of the average of

the checks vs. the average of the fertilizers. This particular breakdown indicates that the greatest difference lies between the fertilizers, as shown by the relatively high mean square for the 5 degrees of freedom in table 2. Although

TABLE 1

Percentage of noxious nitrogen in leaves, crowns, and beets of sugar beet plants receiving various fertilizer treatments

PLOT	TREATMENT	NOXIOUS NITROGEN CONTENT					
		Leaves	Crown Section			Beet	Average
			C_1	C_2	C_3		
29	Check V_2	.62	.46	.28	.21	.16	.35
36	Check V_1	.57	.41	.33	.22	.15	.34
37	Manure	.68	.47	.41	.36	.19	.42
38	Manure Phosphorus Sulfur	.86	.44	.41	.38	.21	.46
39	Gypsum	.72	.67	.31	.21	.27	.44
40	Nitrogen	.88	.46	.36	.30	.18	.44
41	Phosphorus	.50	.34	.28	.21	.13	.29
42	Potassium	.53	.28	.24	.20	.11	.27
Average of checks............		.60	.44	.31	.22	.16	.35
Average of fertilized..........		.70	.44	.33	.28	.18	.39
Average of manured and nitrogen-fertilized................		.81	.46	.39	.35	.19	.44
Average of nonmanured and nonnitrogen fertilized.......		.59	.43	.29	.21	.16	.34

TABLE 2

Analysis of variance for data in table 1

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between locations in the plant....................	4	0.5924
Between samples.................................	40	0.0037
Between treatments.............................	7	0.0532
Between checks..............................	1	0.0006
Between fertilized............................	5	0.0680
Check vs. fertilized...........................	1	0.0313
Treatment × location in the plant..............	28	0.0097
Between checks..............................	4	0.0020
Between fertilized............................	20	0.0123
Check vs. fertilized...........................	4	0.0040
Total...	79	

this evidence is far from conclusive, it would suggest that in a replicated experiment, it may be possible to demonstrate significant differences in levels of noxious nitrogen in the sugar beet plant owing to fertilizer treatment of the soil in which

the beets are grown. If one observes the mean values in table 1 for the various fertilizers, it is obvious that this relatively large mean square for between fertilizers is due to the relatively low values of nitrogen on plots 41 and 42, compared with the high average values on plots 37, 38, 39, and 40.

The interaction between treatment and location in the plant measures the failure of the treatment to have the same effect on the nitrogen content of samples from the various locations of the plant. Twenty-eight degrees of freedom are available for this comparison, but again without replication of the treatments, no valid estimate of error exists to determine whether or not the effect is significant. The mean square is rather low, however, a fact which indicates that the treatment has had the same effect on the percentages of nitrogen in the various parts of the plant. These 28 degrees of freedom can also be broken down into three parts with corresponding divisions of the mean square as shown in table 2.

The average noxious nitrogen in all sections of the beet plants including the leaves is higher in the plants grown with nitrogen fertilizers than in those grown without fertilizers.

TABLE 3

Average noxious nitrogen content of beets of various sizes

AVERAGE WEIGHT OF BEET	RANGE OF NOXIOUS NITROGEN IN BEET	AVERAGE OF NOXIOUS NITROGEN IN BEET
gm.	*per cent*	*per cent*
400 to 500	0.13–0.36	0.23
500 to 600	0.13–0.25	0.18
600 to 700	0.12–0.23	0.16
Above 700	0.12–0.19	0.15

The tendency for the noxious nitrogen of the beet to vary with the fertilizer used has been found by other workers (2, 3, 9, 10, 12). The degree of maturity is probably a greater factor, however, for these workers found that, as beets mature, the percentages of noxious nitrogen tend to decrease. Briem (1) and Herke (5) do not agree on the relationship of the size of the beet to noxious nitrogen content. Our data on this phase of the subject are given in table 3. As the size of the beet increased the percentage of noxious nitrogen in the beet decreased. The quantity is comparatively high in very small beets and decreases as the beets become larger.

The average percentage distribution of the dry matter and noxious nitrogen in the crown sections and in the beets is given in table 4.

Although the percentage of noxious nitrogen is lowest in the beets, 86.18 per cent of the total is contained in the beet and only 4.07 per cent in the crown next to the leaves. The quantity increases slightly as the crown approaches the beet.

Table 5 shows the noxious nitrogen content, in pounds per acre yield, in the various sections of the beet plants.

The fertilizers increased the total number of pounds of noxious nitrogen occur-

ring in the various beet plant sections. This was due primarily to an increased yield of dry matter. In the beets, the noxious nitrogen was greatest where manure was applied to the soil and least where potassium was the fertilizer used.

TABLE 4

Percentage distribution of dry matter and noxious nitrogen in crown sections and in beet of sugar beet plants

PLOT	TREATMENT	CROWN SECTION						BEETS	
		C_1		C_2		C_3		Dry matter	Noxious nitrogen
		Dry matter	Noxious nitrogen	Dry matter	Noxious nitrogen	Dry matter	Noxious nitrogen		
29	Check V_2	1.89	5.35	2.77	4.17	3.21	3.96	92.12	86.52
36	Check V_1	1.49	3.87	2.45	5.08	3.16	4.24	92.90	86.80
37	Manure	1.84	3.79	2.85	5.18	3.63	5.81	91.67	85.20
38	Manure Phosphorus Sulfur	2.23	4.32	3.37	5.92	4.96	8.08	89.45	81.68
39	Gypsum	1.77	4.32	2.61	2.81	3.47	2.65	93.21	90.22
40	Nitrogen	1.74	4.07	2.45	4.68	3.73	5.80	92.08	85.46
41	Phosphorus	0.96	2.44	1.65	3.44	2.41	3.69	94.97	90.42
42	Potassium	1.92	4.43	2.88	5.71	4.07	6.71	91.13	83.14
Average		1.73	4.07	2.63	4.62	3.58	5.12	92.19	86.18

TABLE 5

Noxious nitrogen in leaves, crowns, and beets

In pounds per acre yields under various fertilizer treatments

PLOT	TREATMENT	NOXIOUS NITROGEN				
		Leaves	Crown Section			Beet
			C_1	C_2	C_3	
29	Check V_2	6.0	0.5	0.4	0.4	8.1
36	Check V_1	5.4	0.3	0.4	0.4	6.9
37	Manure	17.2	0.8	1.1	1.2	17.8
38	Manure Phosphorus Sulfur	23.5	0.9	1.1	1.7	17.4
39	Gypsum	8.2	0.6	0.3	0.3	11.9
40	Nitrogen	13.0	0.4	0.5	0.5	8.4
41	Phosphorus	7.4	0.3	0.4	0.7	10.8
42	Potassium	8.9	0.3	0.4	0.5	5.8
Average of checks		5.7	0.4	0.4	0.4	7.5
Average of fertilized		13.0	0.6	0.6	0.8	12.0
Average of all treatments		11.2	0.5	0.6	0.7	10.9

Lyasko (6) found that the noxious nitrogen constituted approximately one third of the total nitrogen of the sugar beet and that 90 per cent of it passed into the molasses. According to our work 29.7 per cent of the total nitrogen in

the beet is noxious nitrogen; 35.0 per cent in crown section C_2; 32.4 per cent in crown section C_3; and 35.7 per cent in the leaves. Hence the noxious nitrogen in the various sections of the sugar beet plant approximates one third of the total. The leaves carry slightly more than the other sections of the plant. Little is gained, so far as the exclusion of noxious nitrogen is concerned, by topping beets at the lowest leaf scar, and it is probable that the loss of sugar is great.

The average total quantity of noxious nitrogen occurring in the crowns of the unfertilized plants approached a constant of 0.4 pound per acre yield. In crown section C_3 of the fertilized beets this was 0.8 pound. Hence the total number of pounds of noxious nitrogen found in the leaves is approximately 10 times that in the three crown sections and is approximately equal to that in the beets. A considerable portion of this noxious nitrogen is betaine, which may give to beet products special value as feed for hogs, beef, sheep, chickens, and turkeys. This same noxious nitrogen may render beet products objectionable as feed for milk cows, inasmuch as betaine and related compounds may give rise to off-flavors in the milk and cream.

SUMMARY

The percentage of noxious nitrogen in sugar beet plants varies with the plant section. It is highest in the leaves, lowest in the beet, and intermediate in the crown. Although not conclusive, the data pointed to the conclusion that fertilizers increase the noxious nitrogen of the various sections of the plant.

The total quantity of noxious nitrogen contained in the beet sections, produced on 1 acre of soil, varied from 0.4 pound in the beet crowns to 11 pounds in the beets, as an average. The leaves carried an average of 10 pounds. These values varied widely, depending on the fertilizer treatment.

The percentages of total nitrogen occurring as noxious nitrogen in the various sections of sugar beet plants varied from 28 in crown section C_1 to 35.7 in the leaves.

Beet crowns and leaves, because of the quantities of betaine which they contain, probably have special nutritive value when fed to hogs, beef, sheep, chickens, and turkeys, but the betaine content may unsuit them as feed for milk cows.

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FUSED TRICALCIUM PHOSPHATE: RELATION OF DEGREE OF DEFLUORINATION TO FERTILIZER VALUE OF QUENCHED FUSIONS OF ROCK PHOSPHATE[1]

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The fertilizer industry had its beginning in the acidulation of bones, but its development has come from the processing of rock phosphate. Both materials have been used extensively as fertilizing phosphates in raw form. It was long assumed that the chief distinction between these two materials was one of phosphate concentration, and the rock was evaluated on the basis of the computed content of "bone phosphate of lime." This misconception prevailed, although the superiority of ground steamed bone over pulverized rock phosphate had been demonstrated conclusively in field trials. Now, however, it is known that the relative insolubility of continental rock phosphates is due to the fact that they are essentially geochemical formations of apatite, or fluorphosphate (8). Bartholomew (2, 3) compared island and continental rocks of variant fluorine content in an effort to correlate such content and P_2O_5 availability in soils of variant degree of acidity.

The fluorides, split off from the apatite of rock phosphate during the manufacture of superphosphate, are retained in large part and were deemed nonreactant in subsequent processings. It has been established, however, that they revert to fluorphosphatic combination in alkaline systems and may induce "reversion," when superphosphates are either ammoniated or processed with liming materials or with defluorinated rock phosphate calcines (1, 7, 14, 15, 16, 17, 18, 19).

THERMAL DEFLUORINATION OF ROCK PHOSPHATE

The extensive studies conducted in the laboratories of the U. S. Department of Agriculture demonstrated that near-complete defluorination of rock phosphate can be effected under certain conditions in the temperature range of 1375-1425°C. and that the resultant calcine has a high degree of P_2O_5 availability (9, 10, 11, 12, 21, 22, 23, 24, 25). Those researches revealed much as to the conditions that promote fluorine removal and convert apatite to a highly available tertiary phosphate. Unfortunately, however, the calcination process has not been implemented commercially.

The concept that the apatite of rock phosphate can be transformed to a readily dissolvable tertiary phosphate through defluorination of the molten rock was developed and implemented by the Department of Chemical Engineering of the

Tennessee Valley Authority (5, 6). A fused-quenched product of proved ferti-
lizer value and admissible as a supplement in livestock feeding is obtained by the
near-complete defluorination of rock phosphate in large-scale operations with
both electric and fuel-heated shaft furnaces at Wilson Dam. A tolerance of
about one-eighth of the initial fluorine content, however, admits of the production
of an effective phosphatic fertilizer at cost considerably below that of complete
defluorination. The Authority therefore sought from cooperative agencies
information and advice as to the influence of particle size and of degree of de-
fluorination upon the fertilizer value of the fused tricalcium phosphate. Ex-
ploratory greenhouse tests indicated that the product should be ground to pass
at least a 50-mesh screen. Since the pot culture comparisons of the present re-
port were directed to the effects of degree of defluorination, the fused-quenched
phosphates of variant fluorine content used were all of less than 100-mesh.

DESIGNATION FOR QUENCHED DEFLUORINATED FUSIONS OF ROCK PHOSPHATE

In the fusion of rock phosphate by the T.V.A. process, the component apatite
is disrupted and a substantial expulsion of fluorine ensues. It will be shown that
the formation of tricalcium phosphate is governed by degree of defluorination.
The defluorinated molten draw is quenched, and the resultant glass-like material
is chiefly the alpha form of tricalcium phosphate. In physical characteristics,
the quenched material is as different from raw rock as coke is from coal. It is
also decidedly different from a defluorinated calcine of rock phosphate, in that
it can be used to enrich ordinary superphosphate without causing loss of P_2O_5
availability, whereas an admixture of the defluorinated powdery calcine induces
substantial percentage reversion. This difference in compatibility has been
ascribed to difference in degree of subdivision (4, 17, 18).

Fusion without defluorination imparts no fertilizer value, and the T.V.A.
product is not merely "fused rock phosphate." That expression and "de-
fluorinated rock phosphate" are no more applicable to the Authority's product
than would be the designation "acidulated rock phosphate" for superphosphate.
The inclusion of the expression "rock phosphate" in the designation of the new
product is deemed inexact and misleading and, therefore, inappropriate. The
designation "fused calcium phosphate" could be applied to other phosphatic
materials and therefore is not adequately specific. The term "fused tricalcium
phosphate" is truly descriptive and therefore is advocated.

EXPERIMENTAL

The several experimental phosphates were subjected to chemical, microscopic,
and x-ray examinations, to Neubauer tests, and to trials in pot cultures of un-
limed, limestoned, and dolomited soils.

Chemical analyses

The partial analyses of the five control phosphates, of the raw rock and its
undefluorinated melt, and of eight quenched fusions[2] of variant degree of de-

[2] For brevity, the term *fusion* will be used to connote the quenched products of variant
degrees of defluorination induced by the fusion of brown rock phosphate.

fluorination are given in table 1. The dense product obtained by the mere melting of the rock was less soluble than the raw rock in ammonium citrate. The effect of degree of defluorination is reflected by the progressive increase in citrate solubility from the minimum of 3.5 per cent for the merely fused material to the maximum of 88 per cent for the completely defluorinated product. The citrate solubility of the fused bone, and also that of the fused tricalcium phosphate derived from the mixture of limestone and H_3PO_4, were less than that of the rock fusion that contained 0.29 per cent of fluorine.

Evaluation of fused tricalcium phosphate by citrate digestion

The fused tricalcium phosphate has not been produced commercially; it has not been defined, and no "official" procedure has been prescribed for its analytical evaluation. A true tertiary phosphate is relatively soluble in an adequate volume of the conventional neutral citrate of 1.09 sp. gr. and pH of 7. Obviously, however, it is untenable and inadmissible to measure the solubility of a full 1-gm. charge of any tertiary form of calcium phosphate by the solid-to-solvent proportion prescribed for the digestion-extraction of those phosphates that remain after the prescribed aqueous extractions of commercial fertilizers. The citrate solution is used to remove any water-insoluble phosphates generated by either processing or analytical washing and is relatively inert upon the small quantity of undissolved rock residues occurring in well-made fertilizers. Only small fractions of the total P_2O_5 content of the acidic fertilizers, therefore, are subjected to the dissolvent action of the citrate. In contrast, a digestion of a 1-gm. charge of fused phosphate imparts a substantial calcium content and alkalinity to the citrate solution, with resultant diminution in its effectiveness as a solvent. A single citrate digestion of a fused tricalcium phosphate can be considered as analogous to the aqueous extraction prescribed as preparatory to the citrate digestion of the water-insoluble content of superphosphates and mixed fertilizers. A successive citrate digestion, therefore, would be upon an analytical residuum more like the one encountered when an analytical charge of a processed fertilizer is subjected to the prescribed aqueous extraction. When subjected to two citrate digestions, a substantially defluorinated fusion registers a P_2O_5 availability approximating that of superphosphate.

When the residuum from a citrate digestion of a 1-gm. charge of the 100-mesh *undefluorinated* quenched rock-fusion was likewise digested, the "availability" value by the two digestions became 6.7 per cent, as against 3.5 per cent for a single digestion. Corresponding dual digestions of the completely defluorinated fusion, S-963, brought a percentage availability of 98.6 per cent instead of the 87.9 per cent value registered by a single digestion.

The determination of citrate-solubility of the defluorinated fusions is affected by particle size and by weight of the charge, whereas these factors are of minor import in the determination of the percentage solubility of the recalcitrant apatite of both raw rock phosphate and its undefluorinated melt. When the 100-mesh fused material of 3.3 per cent fluorine content was ground finer than 300-mesh—the fineness of raw rock "floats"—citrate solubility was increased only 2.3 per cent.

Evaluation of the fusions by carbonated water extraction

Since carbonated water is the dominant solvent in soil systems, it was used as an index of solubility in relation to degree of defluorination.   Charges of the fusions listed in table 1 were weighed into Erlenmeyers, and distilled water, carbonated at 5°C. to 0.03362 normality, then was introduced, the proportion being 0.5 gm. per liter.   The flasks were sealed and their contents agitated end-over-end 2 hours in an insulated chamber at the mean temperature of 15°C. The solution-suspensions then were filtered and P_2O_5 contents of the filtrates

TABLE 1

Partial analysis of the several phosphatic materials

PHOSPHATIC MATERIAL		P_2O_5			CaO	FLUORINE
Lab. no.	Type	Total	Citrate-insoluble*	Available		
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
S-725	Triple superphosphate	51.2	1.7	96.7	21.74	1.63
P-679	Monocalcium	55.6	0.05	99.9	21.41	0.02
S-983	Dicalcium	50.9	0.30	99.4	41.04	0.013
S-819	Bone, fused, quenched	37.8	11.1	70.7	54.38	0.008
S-855	Tricalcium, fused, quenched	45.4	12.4	72.7	51.84	0.04
S-816	Raw brown rock phosphate	33.6	30.5	9.3	45.0	3.45
S-818	"Fusion"	34.4	33.2	3.5	49.3	3.3
S-849	"Fusion"	25.8	21.4	17.1	46.4	1.25
S-854	"Fusion"	30.9	18.1	41.4	42.2	0.86
S-861	"Fusion"	27.9	17.8	36.2	48.2	0.7
S-968	"Fusion"	29.5	11.9	59.7	43.2	0.52
S-859	"Fusion"	28.5	10.6	62.8	45.5	0.4
S-964	"Fusion"	29.1	7.4	74.6	43.4	0.29
S-847	"Fusion"	25.0	4.0	84.0	39.8	0.06
S-963	"Fusion"	28.9	3.5	87.9	43.2	0.00

* By 1-hour digestion of 1-gm. charge, with agitation at 5-minute intervals, in ammonium citrate solution of sp. gr. 1.07, pH of 7.

determined.   The results are shown in figure 1.   The maximal extraction was from the completely defluorinated fusion and accounted for 44.6 per cent of total P_2O_5 content, or one half of the extraction effected by the citrate digestion, whereas the extraction from the undefluorinated melt was slightly less than that from the raw rock.   Defluorination to a 1.25 per cent fluorine content constituted a 62 per cent removal, but the resultant increase in P_2O_5 extraction was less than 10 per cent of the extraction from the completely defluorinated fusion.   Between the points that connote fluorine removals of 62 and 100 per cent, the curve approached a straight line.   The parallel between degree of defluorination and progression of solubility in a solvent as weak and dilute as carbonated water indi-

cates that defluorination beyond 80 per cent affords an effective phosphatic fertilizer.

X-ray powder diffractions of fusions

The x-ray diffraction patterns of the fused phosphates of table 1 and of figure 2 were made by use of a Machlett copper target tube operated 4 hours at 35 kv.
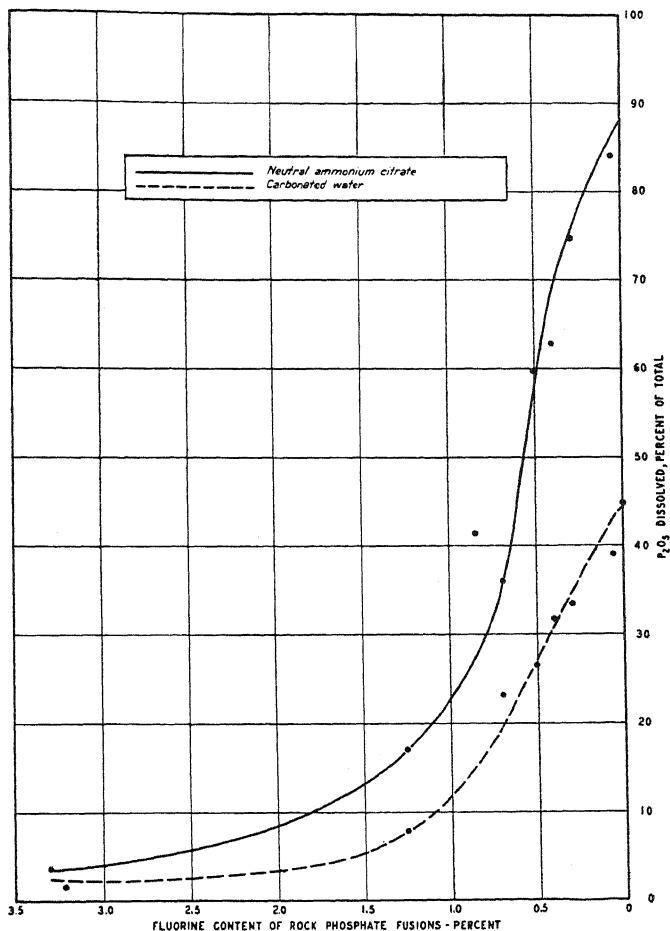
FIG. 1. EFFECT OF DEGREE OF DEFLUORINATION OF ROCK PHOSPHATE FUSIONS ON PROGRESSION IN P₂O₅ SOLUBILITY IN TWO SOLVENTS

and 13 milliamps in the Hayes diffraction unit. A nickel filter was employed to obtain the characteristic $K\alpha$ radiation, $\lambda = 1.539$ Å. Duplitized nonscreen Agfa film was used in twin cameras of circular type and 7-cm. radius, which were calibrated against known spacings of sodium chloride and of calcite. The following results were obtained.

The raw rock, of 3.45 per cent fluorine content, and its melt that contained

3.3 per cent of fluorine, gave strong fluorapatite patterns, identical with the pattern from the Canadian apatite. Fusion S-849, of 1.25 per cent fluorine content, gave a fluorapatite pattern, the lines of which were less intense than those of the raw rock and those of its undefluorinated melt. This intensity decrease indicates the advent of a "glassy" component. Fusions S-854 and S-861, of respective fluorine contents of 0.86 and 0.7 per cent, developed the arrow-demarcated lines for proximate proportions of 25 and 75 for α-$Ca_3(PO_4)_2$ and apatite. Fusions S-968 and S-859, of 0.52 and 0.4 per cent fluorine content,
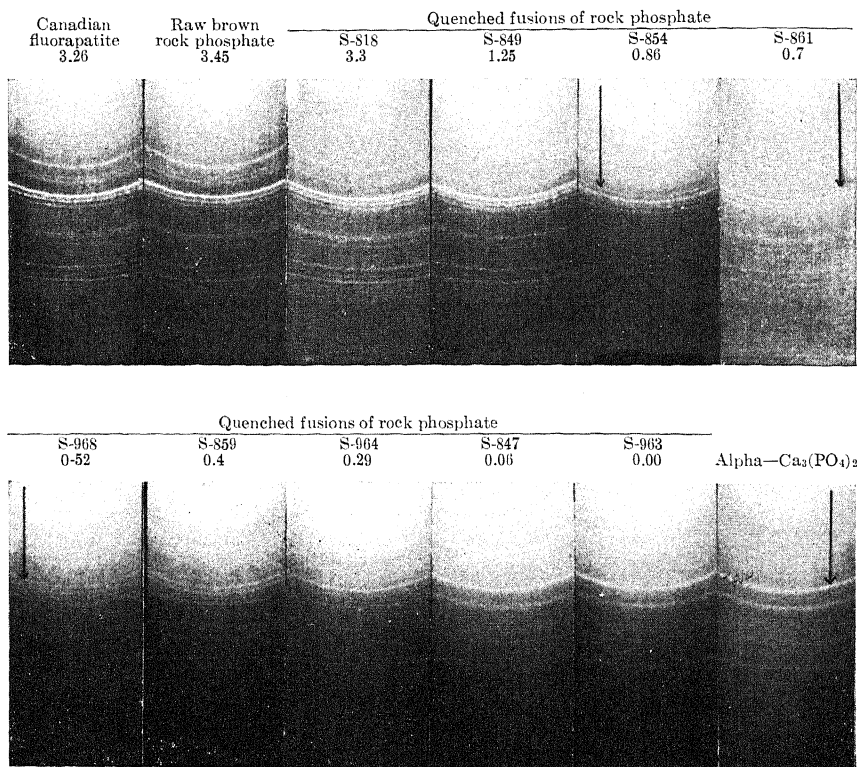
FIG. 2. RELATION OF DEGREE OF DEFLUORINATION TO THE FORMATION OF ALPHA TRICAL-
CIUM PHOSPHATE, AS REGISTERED BY X-RAY POWDER DIFFRACTION
Numerals 3.45 to 0 connote percentage of fluorine content

respectively, developed line intensities that indicated α-$Ca_3(PO_4)_2$, and $Ca_{10}F_2$-$(PO_4)_6$ in proximate proportion of 80 and 20. Fusion S-964, of 0.29 per cent fluorine content, yielded a good pattern of α-$Ca_3(PO_4)_2$, with line weakness indicative of occurrence of some "glassy" material, probably calcium silicate. Fusions S-847 and S-963, of only 0.06 and 0 per cent fluorine content, respectively, gave patterns identical with the patterns of the $Ca_3(PO_4)_2$ control fusion derived from a mixture of limestone and H_3PO_4. The line intensities of alpha tricalcium phosphate increased gradually and those of the fluorphosphate decreased in relation to degree of defluorination.

Optical characteristics of fusions

The optical characteristics of fusions of maximal, intermediate, and minimal fluorine content were determined by means of the immersion technic, with white light and with crossed Nicols. Although the unfused mineral apatite and the undefluorinated fusion, S-818, gave patterns virtually identical, their refractive indexes differed slightly. The particles of the undefluorinated fusion were aggregates of long needle-like crystals, characterized by parallel extinction and low first-order interference colors. The fusion of 1.25 per cent fluorine content showed three distinct types of particles—(a) aggregates of needle-like crystals identical with those of the undefluorinated fusion, (b) aggregates of irregular crystals of anisotropic material, and (c) needles as in (a) in a ground mass of (b). The fusion of 0.85 per cent fluorine content showed two distinctions—(a) the needles present in undefluorinated fusion, and (b) material similar in external appearance to the crystal aggregates occurrent in the fusion of 1.25 per cent fluorine content. The fusion of 0.06 per cent fluorine content was composed of irregularly shaped homogeneous anisotropic aggregates.

P_2O_5 uptake from "fusions" in Neubauer tests

The results of table 2 show the influence of degree of defluorination upon the uptake of P_2O_5 from 25-mgm. additions in two soils, unlimed and limestoned at two rates.

Enhancement in uptake from Hartsells fine sandy loam was virtually identical for the superphosphate, fused bone, fused tricalcium phosphate, and the fusion that contained only 0.06 per cent of fluorine, whereas the uptake from the raw rock, and also that from the undefluorinated melt, was virtually nil. The addition of $CaCO_3$ at the 1500-pound rate increased the uptake from the superphosphate and did not affect the uptake from the fused tricalcium phosphate or that from the fusion that contained only 0.06 per cent of fluorine. The heavier rate of liming repressed the P_2O_5 uptake from the several forms of tertiary phosphate, but did not repress the uptake from the superphosphate. In general, the foregoing effects were registered likewise by Fullerton silt loam, which has been found sensitive to liming rates other than moderate. On both soils, increase in P_2O_5 uptake from the fusions was parallel with increase in degree of their defluorination.

Plant response and P_2O_5 recoveries in pot cultures

The influence of degree of defluorination upon plant response and upon P_2O_5 recovery was determined by three experiments with the two representative Tennessee soils, Hartsells fine sandy loam from the Cumberland Plateau and Fullerton silt loam of the Appalachian Valley, used in the previous experiment. Hartsells is a well-drained soil derived from sand-stone, with a depth of 3 to 4 feet to parent rock. The uneroded surface soil is usually yellowish gray and about 10 inches deep. The subsurface soil of about 20-inch depth is either very fine sandy loam or friable yellow clay loam. Uneroded Fullerton is a brownish gray soil of dolomitic origin and contains cherty fragments to a depth of 8 to 12

inches. The subsurface soil is either yellowish or light red silty clay loam of about 30-inch depth.

Incorporations of K_2SO_4 were made in every experiment, and every nonlegume crop received top-dressings of ammonium nitrate. All rates are expressed in terms of per acre surface and the solid materials were incorporated with the upper 3 inches. All water-insoluble phosphatic materials and the limestone and dolomite incorporations were finer than 100 mesh.

Experiment I. A preparatory, or "robber," crop of Sudan grass was grown 30 days *before* the incorporation of the phosphates and K_2SO_4. This was done to deplete the supply of phosphorus, to allow opportunity for the limestone and dolomite to react with the acidic soils, and to allow the sifted aerated soils to re-

TABLE 2

Effect of degree of defluorination of quenched fusions* of brown Tennessee rock phosphate upon uptake of P_2O_5 in Neubauer tests

PHOSPHATIC MATERIAL		P_2O_5 UPTAKE PER 100 GM. OF SOIL†					
		Hartsells fine sandy loam			Fullerton silt loam		
			Limestoned‡			Limestoned‡	
Lab. no.	Type	Unlimed	1500 lbs.	3000 lbs.	Unlimed	1000 lbs.	2000 lbs.
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
. . . . .	None	−0.4	0.2	0.3	0.3	0.5	−0.2
S-725	Triple superphosphate	7.2	8.3	8.8	9.6	11.2	10.5
S-819	Fused bone	7.5	2.5	1.2	9.7	6.5	2.2
S-855	Fused tricalcium	7.5	7.5	4.7	10.4	8.8	6.7
S-816	Raw rock§	0.4	0.1	0.9	1.4	0.1	0.7
S-818	"Fusion", 3.3% F	0.4	1.1	1.1	1.3	0.7	1.3
S-849	"Fusion", 1.25% F	1.7	2.4	2.8	2.5	2.1	2.4
S-847	"Fusion", 0.06% F	7.3	7.4	5.8	9.1	9.3	7.3

* Less than 100 mesh.
† Net uptake from additions of 25 mgm.
‡ Aging period of 10 days between additions and seeding.
§ Less than 300 mesh.

turn to more normal condition. The "robber" crop then was harvested and the upper 3-inch zone of soil was removed and treated with the several phosphates and K_2SO_4, at respective rates of 40 and 150 pounds of P_2O_5 and K_2O. The initial fertilized crop was seeded in August, 1940. Immediately after the harvesting of this crop the upper 3-inch zone of every pot was removed, conditioned, and returned to the pot without further additions. The second seeding of Sudan grass then was made and the resultant crop was harvested January, 1941. Plant responses to four of the fusions are pictured for the Hartsells and Fullerton soils, respectively, in figures 3 and 4, and all P_2O_5 recoveries are given in tables 3 and 4.

In most cases best response on the Hartsells soil was by the crop sown immediately after the incorporation of the phosphates, and, in general, the phosphate most effective on the initial crop proved to be most effective on the second

crop. The undefluorinated fusion of the rock phosphate exerted no effect upon either plant growth or P_2O_5 recovery. In contrast, plant responses to, and also P_2O_5 recoveries from, the fused product of 0.06 per cent fluorine content and the superphosphate were similar on the Hartsells soil limestoned at the 1500-pound rate, whereas the fused bone and the fused tricalcium phosphate control were less effective in both respects.

On the Hartsells soil limestoned at the rate of 3000 pounds, all of the phosphates, including superphosphate, were less effective, the response of the first crop to the fusion of 0.06 per cent fluorine content being only about half of that

TABLE 3

Yields and P_2O_5 recoveries from 100-mesh "fusions" and controls on limestoned and dolomited Hartsells soil—Experiment I

PHOSPHATES			LIMESTONE—1500 LBS.				LIMESTONE—3000 LBS.				DOLOMITE—3000 LBS.			
			Sudan grass		Total, 2 crops		Sudan grass		Total, 2 crops		Sudan grass		Total, 2 crops	
Lab. no.	Type	Fluorine content	Crop 1	Crop 2	Dry weight	P_2O_5 recovered	Crop 1	Crop 2	Dry weight	P_2O_5 recovered	Crop 1	Crop 2	Dry weight	P_2O_5 recovered
		per cent	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*
.....	None	....	3.7	2.4	6.1	..	3.3	3.1	6.4		4.8	3.1	7.9	
S-725	Triple superphosphate	1.63	16.3	4.4	19.7	22	13.3	6.3	19.4	25	24.9	6.3	31.2	30
S-819	Bone, fused	0.008	11.4	5.4	16.8	17	7.3	7.1	14.4	16	17.4	7.4	24.8	25
S-855	Tricalcium, fused	0.04	13.3	4.9	18.2	19	6.4	5.7	12.1	14	16.9	7.9	24.8	27
S-816	Raw brown rock	3.45	3.6	2.5	6.1	nil	3.8	3.4	7.2	1	5.6	3.2	8.8	2
S-818	"Fusion"	3.3	3.1	1.9	5.0	nil	3.5	4.7	8.2	2	5.0	3.7	8.7	1
S-849	"Fusion"	1.25	5.1	2.2	7.3	2	5.2	5.2	10.4	6	7.3	4.7	12.0	5
S-854	"Fusion"	0.86	11.3	3.1	14.4	11	5.2	4.5	9.7	8	17.8	5.6	23.4	19
S-847	"Fusion"	0.06	15.7	5.2	20.9	21	6.7	8.4	15.1	20	15.9	8.7	24.7	27

to the superphosphate. Plant response and P_2O_5 recovery again were governed by the extent to which the fusions were defluorinated. The second-crop yields and P_2O_5 recoveries from superphosphate were less than those registered by the initial crop.

On the Hartsells soil, dolomite proved more efficacious than limestone in the promotion of growth and in P_2O_5 recovery, when used alone as well as with all of the phosphates. The top response and the highest recovery on the dolomited soil were from the superphosphate. Aggregate yields were virtually the same from the additions of fused bone, fused tricalcium phosphate control, and the rock fusions of 0.86 and 0.06 per cent fluorine content.

On the Fullerton soil, as on the sandy Hartsells soil, plant response and P_2O_5 recovery from the fusions were governed by degree of defluorination. On the unlimed Fullerton soil, the fusion of 0.06 per cent fluorine content proved as effective as superphosphate, whereas the fused bone and the fused tricalcium

TABLE 4

Yields and P_2O_5 recoveries from 100-mesh "fusions" and controls on Fullerton soil, unlimed, limestoned, and dolomited—Experiment I

PHOSPHATES			SUDAN GRASS		TOTAL, 2 CROPS		SUDAN GRASS		TOTAL, 2 CROPS	
Lab. no.	Type	Fluorine content	Crop 1	Crop 2	Dry weight	P_2O_5 recovered	Crop 1	Crop 2	Dry weight	P_2O_5 recovered
		per cent	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*
					Unlimed			*Limestone—1000 lbs.*		
.....	None	....	4.9	2.3	7.2	..	5.8	2.6	8.4	..
S-725	Triple superphosphate	1.63	20.6	3.8	24.4	25	19.8	4.7	24.5	25
S-819	Bone, fused	0.008	22.2	3.9	26.1	27	17.6	3.5	21.1	24
S-855	Tricalcium, fused	0.04	27.8	5.0	32.8	35	20.8	5.0	25.8	30
S-816	Raw brown rock	3.45	7.1	2.9	10.0	6	5.1	3.3	8.4	1
S-818	"Fusion"	3.3	5.8	2.4	8.2	2	5.8	3.1	8.9	nil
S-849	"Fusion"	1.25	10.3	2.7	13.0	7	7.4	2.9	10.3	2
S-854	"Fusion"	0.86	17.9	3.4	21.3	18	16.2	3.8	20.0	18
S-847	"Fusion"	0.06	20.7	4.8	25.5	23	19.9	4.8	24.7	23
					Limestone—2000 lbs.			*Dolomite—2000 lbs.*		
.....	None	....	6.6	3.9	10.5	..	6.1	3.1	9.2	..
S-725	Triple superphosphate	1.63	21.9	6.9	28.8	27	20.6	5.3	25.9	27
S-819	Bone, fused	0.008	14.8	6.7	21.5	22	16.7	4.8	21.5	24
S-855	Tricalcium, fused	0.04	17.2	8.3	25.5	26	17.1	5.6	22.7	26
S-816	Raw brown rock	3.45	6.7	3.3	10.0	1	6.2	2.8	9.0	nil
S-818	"Fusion"	3.3	7.3	3.5	10.8	1	7.1	2.9	10.0	1
S-849	"Fusion"	1.25	8.5	4.2	12.7	4	8.6	3.5	12.1	5
S-854	"Fusion"	0.86	11.6	4.7	16.3	12	14.0	3.9	17.9	14
S-847	"Fusion"	0.06	15.9	7.3	23.2	21	17.4	5.2	22.6	24

phosphate controls gave better crop yields and P_2O_5 recoveries. With limestone at the 1000-pound rate, the fused tricalcium phosphate control, S-855, again induced better growth and higher recovery than did either superphosphate or the fusion of low fluorine content, the two last mentioned materials being of comparable effect. With increase in liming rate to 2000 pounds, the superphosphate

proved more effective, whereas the low fluorine fusion became less effective. Dolomite was less repressive than limestone upon the effectiveness of the fused materials. Since the Fullerton soil is of dolomitic origin, it did not give to additive magnesium the response usually shown by the Hartsells soil.
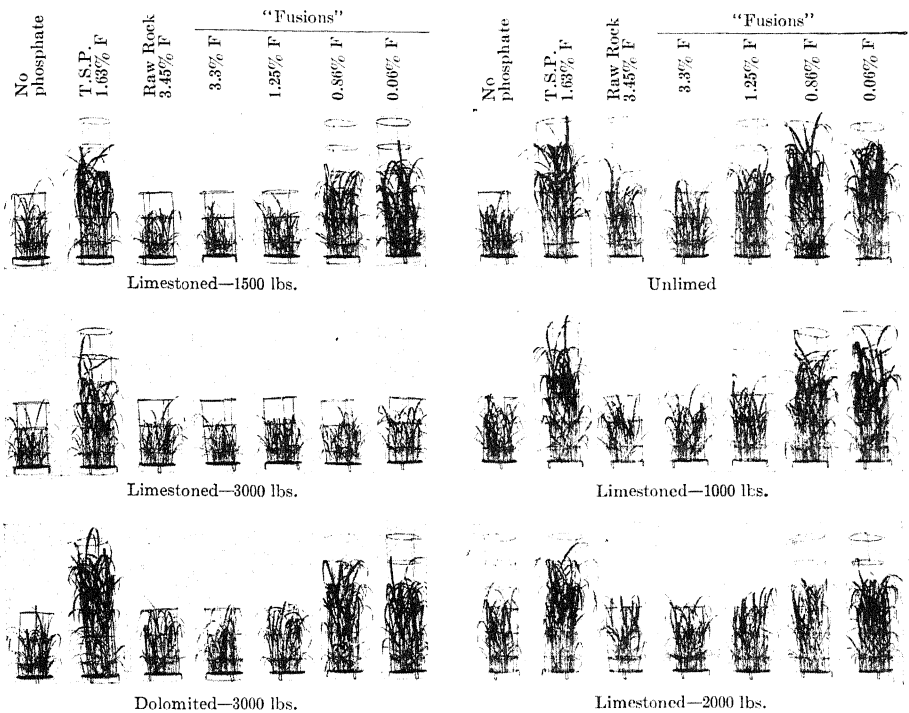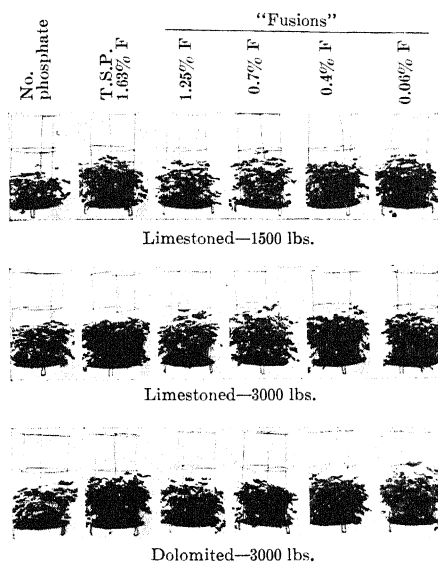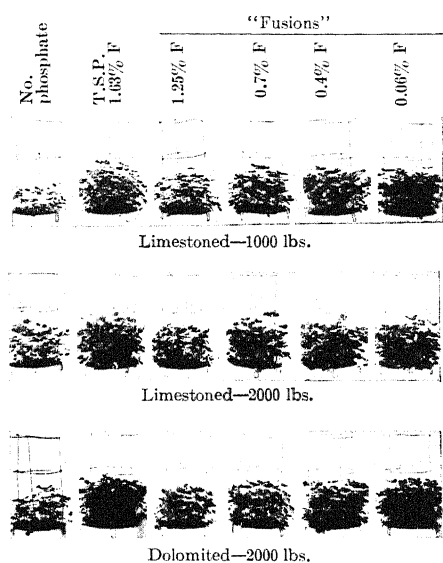

FIG. 3

FIG. 4

FIG. 3. RESPONSE BY SUDAN GRASS TO 40-POUND ADDITIONS OF P_2O_5 AS 100-MESH FUSIONS OF VARIANT FLUORINE CONTENT—HARTSELLS SOIL

Left to right—no phosphate; triple superphosphate, 1.63 per cent fluorine; raw rock phosphate, 3.45 per cent fluorine; fusions, 3.3, 1.25, 0.86, and 0.06 per cent fluorine.

FIG. 4. RESPONSE BY SUDAN GRASS TO 40-POUND ADDITIONS OF P_2O_5 AS 100-MESH FUSIONS OF VARIANT FLUORINE CONTENT—FULLERTON SOIL

Left to right—no phosphate; triple superphosphate, 1.63 per cent fluorine; raw rock phosphate, 3.45 per cent fluorine; fusions, 3.3, 1.25, 0.86, and 0.06 per cent fluorine.

The results of experiment I demonstrate that defluorination beyond 75 per cent is imperative to impart to the fusions a substantial enhancement in fertilizer value.

Experiment II. It has been demonstrated that clover will not mature on greenhouse cultures of the unlimed Hartsells and Fullerton soils. Composites of the several limed and twice-cropped cultures of experiment I, therefore, were used in red clover cultures in experiment II, which was amplified to include fourfold and eightfold incorporations of P_2O_5 as raw rock and as its undefluorinated fusion. The phosphates and the potassium sulfate were incorporated at the

rates and in the manner described for experiment I. The upper 3-inch soil zone also received a 40-pound incorporation of MgO as basic magnesium carbonate and a composite solution of salts of boron, copper, manganese, and zinc prior to the seeding in February, 1941. Plant responses are pictured in figures 5, 6, and 7, and P_2O_5 recoveries are recorded in table 5.

TABLE 5

Yields and P_2O_5 recoveries from 100-mesh "fusions" and from raw and undefluorinated fused rock by red clover on two soils—Experiment II

Lab. no.	Type	Mesh	Fluorine content	P_2O_5 per acre	Hartsells Fine Sandy Loam Limestone 1500 lbs. Dry wt.	P_2O_5 recovered	Limestone 3000 lbs. Dry wt.	P_2O_5 recovered	Dolomite 3000 lbs. Dry wt.	P_2O_5 recovered	Fullerton Silt Loam Limestone 1000 lbs. Dry wt.	P_2O_5 recovered	Limestone 2000 lbs. Dry wt.	P_2O_5 recovered	Dolomite 2000 lbs. Dry wt.	P_2O_5 recovered
			per cent	lbs.	gm.	per cent	gm.	per cent	gm.	per cent	gm.	per cent	gm.	per cent	gm.	per cent
.....	None	...	....	..	5.2	..	9.8	..	8.6	..	2.3	..	6.4	..	5.4	..
S-725	Triple superphosphate	20	1.63	40	14.7	9	18.7	13	20.0	11	14.2	14	16.1	13	17.7	15
S-816	Raw brown rock	325	3.45	160	8.0	4	12.1	1	11.3	1	6.1	5	9.2	3	7.8	2
S-816		325	3.45	320	9.3	5	13.1	4	12.3	3	10.0	6	9.4	4	10.6	lost
S-818	Fused brown rock	100	3.3	160	7.2	3	11.3	nil	10.1	nil	4.5	3	6.6	nil	6.7	nil
S-818		100	3.3	320	7.3	3	12.4	3	9.9	nil	5.0	4	8.2	2	6.8	1
S-849	"Fusion"	100	1.25	40	8.2	4	14.6	4	12.2	1	6.8	6	8.9	3	8.1	1
S-861	"Fusion"	100	0.7	40	11.0	6	16.1	5	14.2	5	9.4	9	11.7	6	11.1	5
S-859	"Fusion"	100	0.4	40	13.8	8	17.7	11	17.8	9	15.9	15	14.8	11	15.4	11
S-847	"Fusion"	100	0.06	40	16.2	11	16.6	9	17.1	9	14.7	15	15.0	12	14.7	11

* From the same rock. Additions equivalent to 40 lbs. of P_2O_5 per acre surface. Potassium sulfate was supplied to all pots at the per acre rate of 150 lbs. of K_2O.

Responses and recoveries were comparable for superphosphate and the fusions of low fluorine content on the lightly limestoned soils. Increase in liming rates repressed slightly the effectiveness of the fusion of low fluorine content, but did not exert a similar effect upon the fusions of intermediate fluorine content. Greater degree of defluorination imparted an increase in the fertilizer effectiveness of the fusions. The fusion that contained 1.25 per cent of fluorine proved decidedly less effective than the three fusions of lower fluorine content, as would be expected from the dominance of apatite indicated by the x-ray pattern and in harmony with solubilities registered by citrate and carbonated water extrac-

tions. The effectiveness of the fusion that contained 0.4 per cent of fluorine was virtually equal to that of the one that contained only 0.06 per cent.

Even when the raw rock and its undefluorinated fusion were used to supply P_2O_5 at fourfold and eightfold rates, these materials were generally ineffective and gave little or no P_2O_5 recovery on the soils that were either limestoned or dolomited.

FIG. 5. RESPONSE BY RED CLOVER TO 40-POUND ADDITIONS OF P_2O_5 AS 100-MESH FUSIONS OF VARIANT FLUORINE CONTENT—HARTSELLS SOIL

Left to right—no phosphate; triple superphosphate, 1.63 per cent fluorine; fusions, 1.25, 0.7, 0.4, and 0.06 per cent fluorine

FIG. 6. RESPONSE BY RED CLOVER TO 40-POUND ADDITIONS OF P_2O_5 AS 100-MESH FUSIONS OF VARIANT FLUORINE CONTENT—FULLERTON SOIL

Left to right—no phosphate; triple superphosphate, 1.63 per cent fluorine; fusions, 1.25, 0.7, 0.4, and 0.06 per cent fluorine

Experiment III. Four crops—red clover, soybeans, Sudan grass, and red clover—were grown in that succession on Hartsells and Fullerton soils between February, 1942, and May, 1943, for comparisons between fusions that contained 0.52, 0.29, and 0.0 per cent of fluorine and equivalent incorporations of monocalcium and dicalcium phosphates. In this experiment, however, the soils were limestoned to a depth of 6 inches at the respective rates of 4500 pounds and 2500 pounds of $CaCO_3$ per acre surface. After 1 month of aging, K_2SO_4 was incorporated likewise to supply K_2O at the 100-pound rate. The phosphates then were incorporated with the upper 3-inch zone and the pots were seeded immediately.

The P_2O_5 depletion by each harvest of the first crop was restored, along with a second 40-pound addition before the second seeding. Neither restowals nor

FIG. 7

FIG. 8
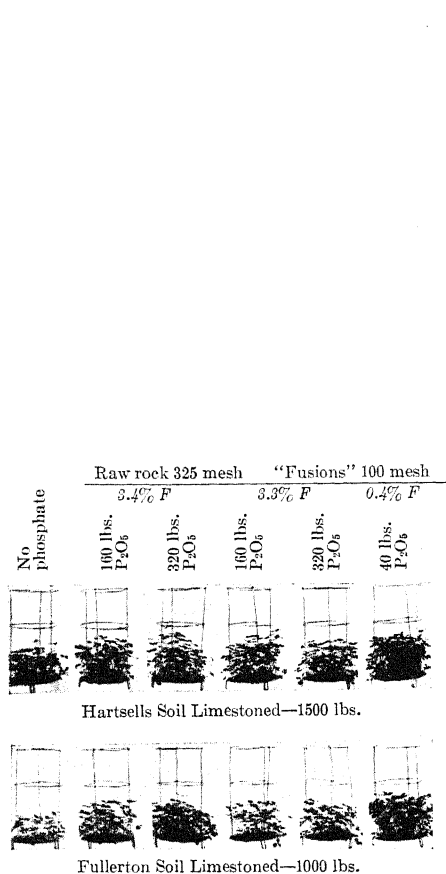
FIG. 7. RESPONSE BY RED CLOVER TO ROCK PHOSPHATE AND TO FUSIONS OF VARIANT FLUORINE CONTENT

Left to right—no phosphate; raw rock phosphate, 325 mesh, 3.4 per cent fluorine, 160 lbs. P_2O_5; raw rock phosphate, 325-mesh, 3.4 per cent fluorine. 320 lbs. P_2O_5; fusion, 100-mesh, 3.3 per cent fluorine, 160 lbs. P_2O_5; fusion, 100-mesh, 3.3 per cent fluorine, 320 lbs. P_2O_5; fusion, 100-mesh 0.4 per cent fluorine, 40 lbs. P_2O_5.

FIG. 8. COMPARATIVE RESPONSE TO MONOCALCIUM AND DICALCIUM PHOSPHATES AND TO FUSIONS OF VARIANT FLUORINE CONTENT

Left to right—no phosphate; monocalcium phosphate, 0.02 per cent fluorine; dicalcium phosphate, 0.013 per cent fluorine; fusions, 0.52, 0.29, and 0 per cent fluorine.
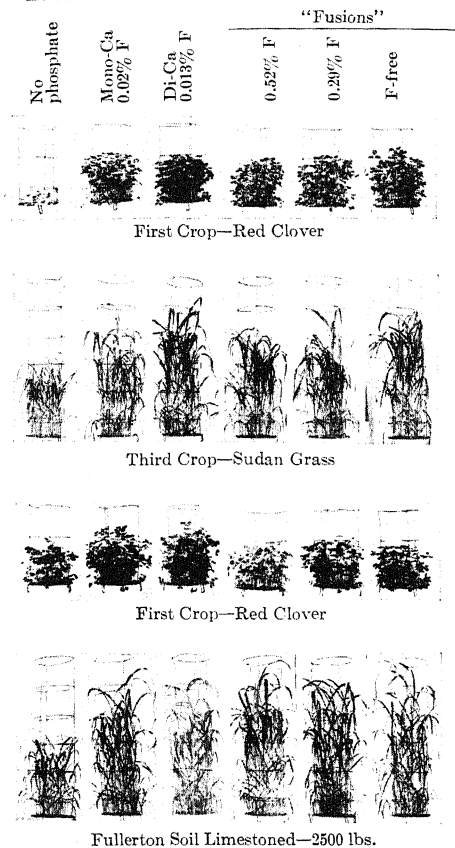
additions of P_2O_5 were made to the third crop, Sudan grass, but 40-pound repetitions were made before the seeding of the fourth crop. Additions of potassium sulfate were made, however, in advance of all of the four seedings, and ammonium

nitrate was applied to the Sudan grass. All pots also received boron, copper, zinc, manganese, and magnesium in appropriate quantities. The first and third crops from both soils are pictured in figure 8 and the P_2O_5 recoveries are given in table 6.

On the Hartsells soil the aggregate yields from the three fusions were respectively equal to or greater than those from the monocalcium phosphate, and two of the recoveries of P_2O_5 were equal to the recovery from the monocalcium phos-

TABLE 6

Yields and P_2O_5 recoveries from 100-mesh "fusions" by four successive crops on two limestoned soils—Experiment III

PHOSPHATES			HARTSELLS FINE SANDY LOAM				Total, 4 crops		FULLERTON SILT LOAM				Total, 4 crops	
Lab. no.	Type	Fluorine content	Red clover*	Soy beans†	Sudan grass‡	Red clover	Dry wt.	P_2O_5 recovered	Red clover*	Soy beans†	Sudan grass‡	Red clover	Dry wt.	P_2O_5 recovered
		per cent	gm.	gm.	gm.	gm.	gm.	per cent	gm.	gm.	gm.	gm.	gm.	per cent
.....	None	....	0.6	12.8	5.6	2.9	21.9	....	6.0	18.5	7.6	5.4	37.5	....
P-679	Monocalcium	0.02	11.1	20.0	10.3	14.2	55.6	26.0	11.9	25.8	11.1	16.2	65.0	40.0
S-983	Dicalcium	0.013	13.6	22.0	11.5	18.6	65.7	29.0	15.2	23.6	13.8	17.4	70.0	28.0
S-968	"Fusion"	0.52	9.6	23.0	9.0	13.8	55.4	22.0	8.7	20.8	11.9	14.6	56.0	20.0
S-964	"Fusion"	0.29	10.7	22.3	9.6	15.4	58.0	30.0	11.3	21.2	13.8	16.6	62.9	29.0
S-963	"Fusion"	0.00	12.2	23.3	11.4	16.3	63.2	26.0	10.2	22.7	13.5	17.4	63.8	27.0

* Incorporations were at the rate of 40 lbs. of P_2O_5 per acre surface to the two seedings of red clover and the soybeans. Potassium sulfate additions at the 100-pound K_2O per acre rate were made before each of the four seedings.

† The respective removals of P_2O_5 by the initial crop of red clover were restored with the other supplements.

‡ Neither restorals nor additions of phosphates were made when the Sudan grass was seeded.

phate. The highest response was that from the dicalcium phosphate, and the P_2O_5 recoveries from it and from the fusion of 0.29 per cent fluorine content were virtually identical and maximal.

On the Fullerton soil the largest aggregate response was induced by the dicalcium phosphate, whereas maximal P_2O_5 recovery was from the monocalcium phosphate. The aggregates of plant response from the two fusions of lowest fluorine content were close to the aggregate from the monocalcium phosphate, whereas P_2O_5 recoveries from these two fusions were virtually the same as the recovery from dicalcium phosphate.

DISCUSSION

In pursuit of a major objective, T.V.A. has developed processes for the manufacture of concentrated phosphatic materials, and substantial tonnages of several new products have been produced and distributed for tests by cooperative experimental agencies. One of these products is fused tricalcium phosphate. The effect of particle size has been studied in plot and field tests conducted at several experiment stations, the findings of which have not been published.[3] From comparisons in pot cultures, Jacob and Ross (12) concluded that, when finer than 80-mesh, fused tricalcium phosphate of 0.12 per cent fluorine content was "approximately equal to superphosphate . . . for the growth of plants on acid and neutral soils." From greenhouse studies with four crops on two loams, Karraker et al. (13) concluded that fusions of undisclosed fluorine content and superphosphate were of equal value. In their summary of results from field tests in Kentucky, they placed the value of the fused tricalcium phosphate above that of superphosphate on both limed and unlimed soils.

In a program that includes the growing of legumes in soils of the humid Valley Area, the utility of phosphates should be considered in relation to rational liming. In the studies of the present report, this factor was recognized by the use of moderate supplements of limestone and dolomite. Exploratory Neubauer tests and pot cultures demonstrated that the effectiveness of the new product was governed by particle size. Nevertheless, the difference between the effectiveness of 100-mesh material and that of a 50-mesh sifting was not sufficient to warrant the extra cost of grinding to a fineness of less than 100 mesh. Since the primary objective of the present study was to determine the influence of degree of defluorination upon availability, all solid materials, other than superphosphate, were of 100-mesh fineness.

From determinations of solubility in ammonium citrate and in carbonated water, Neubauer tests, and pot studies, it was found that progression in the availability of the P_2O_5 content of the 100-mesh quenched fusions of brown rock is governed by degree of defluorination. The studies establish the fact that a 36 per cent fluorine removal imparts only meager increase in P_2O_5 solubility, that 80 per cent defluorination should be attained, and that diminution of fluorine content to 0.4 per cent imparts a satisfactory degree of P_2O_5 availability.

The x-ray patterns demonstrate that the enhancement in P_2O_5 availability resultant from the thermal defluorination of rock phosphate and quenching of the fusions is attributable to the transition of apatite to alpha tricalcium phosphate. They indicate also that approximately 85 per cent of the P_2O_5 content of a fusion carrying 0.4 per cent of fluorine is in that combination and that the other 15 per cent is accounted for by undisrupted apatite, which is disseminated throughout the body of readily dissolvable tricalcium phosphate. When the more soluble tertiary component of the fusions undergoes dissolution in the soil, the less soluble residual apatite probably is dispersed in a state of subdivision far beyond

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[3] The contribution by Alway, F. J., and Nesom, G. H., *Journal American Society of Agronomy*, January 1944, appeared subsequent to the completion of the present paper.

that attainable by the comminution of the raw rock. This may be one reason why the fusion of 0.4 per cent fluorine content registered a fertilizer effectiveness virtually the same as that registered by the fusion that contained less than 0.1 per cent of fluorine. A good crop, however, seldom removes more than 30 per cent of a 40- to 50-pound addition of P_2O_5. Since the crop requirements were met by the quantity of P_2O_5 carried by experimental incorporation of the fusion that contained 0.4 per cent of fluorine, this fusion proved virtually as effective as the somewhat more readily dissolvable fusion of lower fluorine content. Because of the chemical and physical properties of the fused tricalcium phosphate, it was deemed essential that the added fusions be distributed throughout the stipulated zones of soil in the pot cultures. Since band drilling is not feasible in the potted soils, the relative merits of methods of incorporation have not been considered.

CONCLUSIONS

The relationship between degree of defluorination and enhancement in the P_2O_5 availability of quenched fusions of brown rock phosphate was registered by solubility in two solvents, by Neubauer tests, and by crop response and P_2O_5 recovery in pot cultures. An 80 per cent removal of initial fluorine content is deemed necessary to impart a satisfactory increase in fertilizer value. X-ray diffraction patterns demonstrated that a substantially defluorinated and quenched fusion of rock phosphate is composed chiefly of the readily available alpha form of tricalcium phosphate.

The present findings indicate that a quenched rock phosphate fusion carrying not more than 0.4 per cent fluorine and of 100-mesh fineness is a satisfactory fertilizer for *incorporation* with neutral soils, those mildly acidic, and those limestoned or dolomited to the extent that assures the growing of red clover. Because of the similarity of results obtained in previous related comparisons of separates of substantially defluorinated and quenched fusions finer than 50-mesh and finer than 100-mesh, the foregoing statement is deemed appropriate also for material finer than 50-mesh.

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HYDROGEN-ION CONCENTRATION OF THE IMPORTANT SOILS OF THE UNITED STATES IN RELATION TO OTHER PROFILE CHARACTERISTICS: I. PEDOCAL SOILS

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For more than two decades much attention has been given to the hydrogen-ion concentration of soils in various countries of the world. In the earlier work attention was given primarily to surface soils and to the effects of various cultural treatments. More recently interest in various profile characteristics has led to consideration of soil acidity at various depths. During the progress of the soil survey of the United States, the Division of Soil Survey has had occasion to determine the pH values of many carefully selected soil samples. It seems desirable to bring together in one publication the more useful of such data, and to relate them to the great soil groups and to many of the important soil series of the United States.

HISTORICAL RÉSUMÉ

In view of the fact that soil literature contains such a large mass of data dealing with hydrogen-ion concentration, it seems desirable to make a brief summary of some of the literature dealing with the subject on the basis of the natural soil profile. Such résumé necessarily eliminates a very considerable part of all the work done, since the profile aspect has not been extensively considered. The purpose at this time is not to make a complete review but rather to draw attention to a few of the contributions to literature that emphasize general pH characteristics of several of the more important soil groups throughout the world.

The pH characteristics of the podzol soils are perhaps the best known by soil investigators. These profiles are normally strongly acid throughout the solum. Among the investigators that have noted this characteristic in different parts of the world may be mentioned Jenny (36), of Switzerland; Stebbut (54), of Yugoslavia; Smolik (53), of Czechoslovakia; Mattson and Gustaffson (45), of Sweden; Björlykke (3), of Norway; and various publications of the U. S. Department of Agriculture dealing with soils of the United States (7, 10).

Brown podzolic soils and gray-brown podzolic soils have been widely studied, but their characteristics are much more varied than are those of typical podzols. In general they are not so strongly acid as the podzols. The greatest acidity usually occurs in the lower A or upper B horizons. In addition to some of the investigators mentioned, several others have emphasized the characteristics of the gray-brown podzolic profiles. Among these are Mitchell and Muir (46), in Scotland, and various workers in the United States (7, 10, 12, 13, 33, 35).

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[1] Formerly of the Division of Soil Survey, Bureau of Plant Industry, Soils and Agricultural Engineering; now of the Grain Products Branch, Office of Distribution, War Food Administration.

The pH values of red and yellow podzolic soils in various parts of the world have been mentioned. Thorp and associates (66, 67) deal with these groups in China; Liatsikas (43), in Greece. DeSigmond (14) also mentions soils from various parts of the world that are probably related to this group. Red and yellow podzolic soils show but little difference in pH characteristics from those of the gray-brown podzolic group. In general, lateritic soils of the United States and of island possessions and other parts of the world show similar pH characteristics. In this connection should be mentioned the work of Bonnet (4) in Puerto Rico, and Hornby (34) in South Central Africa.

Chernozem soils have an important part in European agriculture. The pH values of profile samples of such soils have been reported from various parts of this continent. Among them may be mentioned the works of Radu, Till, De Sigmond, and others (14, 50, 65, 68). Wyatt and Newton of Canada have described soils of that country (75) including various profiles of chernozems, and pH values of various profiles of United States soils have appeared in different publications (5, 33). In general, it may be said that in this group of soils the surface layers are very slightly acid or neutral. Though lower layers are calcareous, the pH values do not often exceed a pH of about 8.5.

The various groups of soils found in regions of low rainfall have been reported. In general, the pH values of soils formed under dry land conditions vary from neutral to very strongly alkaline, depending in large measure upon moisture relations and parent materials. Values higher than 8.5 usually reflect the presence of sodium carbonate or other alkaline salts accumulated under poor drainage conditions.

Solonetz profiles reported by Storie (55), Shaw and Kelley (52), and Kellogg (37) show great extremes; they vary from medium-acid in the A horizon to very strongly alkaline in the B and C horizons.

A brief sketch of existing literature dealing with the pH values of soil profiles covers only part of the outstanding literature but will serve to furnish a fairly good key to the more general references of this character. Some of these references will be cited again in this series of papers in comparison with specific profiles in the United States, and others not mentioned here will also be given brief consideration. This series deals primarily with unpublished data for soils of continental United States, supplemented by a small portion of the information already published in Soil Survey reports of various counties and areas throughout this country.

The plan of work involves the placing of a great deal of emphasis on proper identification of the soil types and the collection of numerous soil samples on the basis of the natural soil profiles. Hydrogen-ion determinations are then made and the pH data are correlated with various morphological features involved in soil identification and classification. The soil samples collected are representative of all the important great groups of mineral soils in continental United States and many of the important soil series. Whenever possible at least three profiles for each series have been selected. In some places the profile samples were collected in road cuts where the preparation of vertical soil faces was relatively

easy; in other places it was necessary to dig pits from which samples were taken out of the exposed vertical faces. Individual horizon samples were unmixed with material of other horizons. The soil samples, which of course varied in moisture content as taken, were placed in small canvas bags and allowed to air-dry in transit to Washington. These samples, after varying periods of drying, were gently rolled to pass a 2-mm. sieve in accordance with conventional procedure.

The pH determinations were made by means of a hydrogen electrode, in the manner described in a previous publication (1). One part of soil was treated with two parts of distilled water by volume, shaken for a short time, and transferred to a bubbling type hydrogen electrode vessel. The voltage readings were taken when equilibrium was reached, which required 5 to 10 minutes. Duplicate determinations were made of each sample. It is well known that moist soil contains varying quantities of bicarbonates in equilibrium with carbon dioxide

TABLE 1

Terms used to describe the reaction of soils of known pH*

DESIGNATION	pH
Extremely acid	Below 4.5
Very strongly acid	4.5–5.0
Strongly acid	5.1–5.5
Medium-acid	5.6–6.0
Slightly acid	6.1–6.5
Neutral	6.6–7.3
Mildly alkaline	7.4–8.0
Strongly alkaline	8.1–9.0
Very strongly alkaline	9.1 and higher

* From Soil Survey Manual (39).

of the soil atmosphere. The bubbling action with hydrogen served to remove a considerable part of the carbon dioxide gas and thus tended to decrease acidity. Comparison of results with those obtained with methods where bubbling was not involved, however, showed negligible differences except in cases where soils were water-logged.

In view of the fact that it is frequently necessary to compare in a general way the acidity and alkalinity of different soils, table 1 has been prepared. This shows each of the normally used designations of relative acidity.

CLASSIFICATION OF SOILS

The soil profiles selected for this series of papers are classified according to the system in use by the Bureau of Plant Industry (70). All soils are grouped into three main divisions: zonal, intrazonal, and azonal. The zonal soils may be divided into pedocals, pedalfers, and transitional. The pedocals are roughly equivalent to the soils of the subhumid, semiarid, and arid regions. The pedalfers are roughly equivalent to soils of the humid regions. The transitional soils are intermediate between the pedocals and the pedalfers.

The acidity of soils does not readily lend itself to cartographic expression, since differences in pH within a single profile are often great and because soil types of greatly different pH values may occur within small areas. In view of the desirability for concise expression of extensive data in these papers, a map showing the United States has been constructed to include the location of profiles set forth in detail in the vaious tables. The location of each profile on the map is indicated by a number, which corresponds to the number in some one of the tables presented.

In several areas the profiles studied are closely bunched, and other areas are bare of profile locations. This was unavoidable, as the samples were collected and most of the determinations were made not specifically for this series of papers, but rather for the correlations of soil survey areas. In the bare areas either no samples had been collected or the collections, made years ago, had not been by horizons, and therefore the samples were unsuitable for accurate studies.

This paper deals with the hydrogen-ion concentration of the pedocal soils. These include the dark-colored soils of the subhumid and semiarid grasslands, consisting of the great soil groups known as chernozems, chestnut soils, and reddish chestnut soils, and the light-colored soils of arid regions, including the following great soil groups: brown soils, reddish brown soils, sierozems, desert soils, and red desert soils. Calcification (38) is the dominant soil-forming process under which these soils, especially the chernozems and the chestnut and reddish chestnut soils, are produced. This is evidenced by their only slightly acid to strongly alkaline upper horizons (tables 2 to 9).

CHERNOZEMS

Three soil series were selected as typical of the chernozem soils in the United States (fig. 1) (23, 38, 44, 70). They are the Barnes (20, 40, 73, 74) Moody (20, 25, 51, 73), and Holdrege (9, 30, 31, 69).

The pH values of these profiles vary from 5.9 to 8.7 (table 2). Of the 14 profiles studied, only 1 has any horizon above the lime zone that is of medium acidity, 7 have some upper horizons that are slightly acid, the upper horizons in 5 profiles are neutral, and in 1 mildly alkaline (tables 1, 2).

In every profile but one, the lime zone is more alkaline than any horizon above it. In this one exception (profile 2), the horizon just above the lime zone has the same pH. Only 4 out of the 14 profiles have horizons more alkaline than 8.5. These are found in the lime zone or below it. A reaction above 8.5 indicates the presence of sodium or potassium carbonate.

The soils of all three series have vitually the same range of pH values throughout their profiles. One Holdrege profile in its subsurface horizon has a pH of 5.9, the lowest of any of the chernozem soils examined. The horizons above the lime zone in the Moody average a trifle lower in pH values than those of the Barnes, and those of the Holdrege average a trifle lower than those of the Moody. The pH of the compact zone is intermediate between that of the lime zone below it and that of the horizon above it.

TABLE 2

Chernozems

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Barnes loam	
1	Lac Qui Parle Co., Minn.	0–8	1. Dark brown to black loam	6.2
		8–18	2. Brown to dark brown heavy loam or clay loam	7.2
		18–28	3. Light yellow brown heavy clay loam to silty clay	7.1
		28–40	4. Lime zone. Light yellow brown to grayish yellow silty clay with some sand and pebbles	8.2
2	Cass Co., N. Dak.	0–14	1. Dark gray to almost black loam	7.1
		14–20	2. Dark brown loam, becoming lighter colored and heavier textured with depth	7.9
		20–55	3. Lime zone. Pale brownish yellow passing into greenish yellow light friable clay	7.9
3	Kingsbury Co., S. Dak.	0–2	1. Very dark brown granular loam	8.1
		2–11	2. Very dark brown granular loam	7.8
		11–23	3. Brown heavy loam	7.4
		23–44	4. Lime zone. Grayish yellow loam	8.4
		44–53	5. Grayish yellow loam	8.6
		53–64	6. Grayish yellow clay loam with some sand and pebbles	8.7
		64–73	7. Gray clay loam with some sand and pebbles	8.7
			Barnes silt loam	
4	Brown Co., S. Dak.	0–14	1. Almost black silt loam	6.7
		14–24	2. Brown silt loam	7.6
		24–28	3. Grayish yellow silty clay loam	7.3
		28–38	4. Lime zone. Grayish yellow silty clay loam	7.9
		38–54	5. Yellow silty clay loam mottled with red, brown, and drab	8.2
5	Moody Co., S. Dak.	0–2½	1. Dark brown silt loam	6.7
		2½–8	2. Almost black light silt loam	6.4
		8–23	3. Brown silt loam	7.1
		23–47	4. Lime zone. Grayish yellow loam	8.3
		47–60	5. Grayish yellow loam	8.5
		60–66	6. Grayish yellow clay loam	8.5
6	Cedar Co., Nebr.	0–1½	1. Platy layer, nearly black silt loam	6.1
		1½–12	2. Very dark grayish brown to black silt loam	6.3
		12–20	3. Grayish brown heavy silt loam	6.7
		20–40	4. Yellowish brown silt loam	7.1
		40–56	5. Lime zone. Light yellowish brown light silt loam	8.4
		56–65	6. Grayish brown loess	8.6
		65–72	7. Light grayish brown medium sand	8.6

TABLE 2—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Barnes silt loam—Continued	
7	Colfax County, Nebr.	0–1½	1. Very dark grayish brown granular silt loam	7.3
		1½–12	2. Very dark grayish brown granular silt loam	6.9
		12–19	3. Grayish brown granular silty clay loam	7.1
		19–40	4. Lime zone. Grayish brown structureless silt loam	8.1
		40–58	5. Lime zone. Gray structureless silt loam	8.1
		58–96	6. Parent loess, gray structureless silt loam	8.0
8	Lac Qui Parle Co., Minn.	0–2	1. Very dark grayish brown silt loam	7.2
		2–10	2. Dark grayish brown silt loam	6.2
		10–21	3. Grayish brown silt loam	6.7
		21–29	4. Lime zone. Grayish yellow silt loam with light gray spots	8.5
		29–37	5. Grayish yellow heavy silt loam	8.4
		37–59	6. Brown yellow or gray heavy silt loam	8.3
		59–62	7. Gray silty clay loam	8.3
9	Moody Co., S. Dak.	0–3	1. Almost black silt loam	6.2
		3–12	2. Almost black silt loam	6.4
		12–20	3. Dark brown silt loam	6.3
		20–30	4. Nearly black and brown mottled silt loam	6.6
		30–35	5. Yellow silt loam	7.2
		35–41	6. Lime zone. Grayish brown silt loam	8.2
		41–49	7. Lime zone. Grayish brown silt loam	8.2
		49–63	8. Lime zone. Mottled red and gray silt loam	8.2
		63–77	9. Yellow and gray mottled silt loam	8.0
		77–107	10. Yellow clay	8.1
10	Moody Co., S. Dak.	0–4	1. Dark brown silt loam	7.4
		4–11	2. Nearly black silt loam	7.0
		11–20	3. Brown silt loam	6.8
		20–34	4. Yellow silt loam	7.3
		34–44	5. Lime zone. Yellow silt loam	8.7
		44–56	6. Lime zone. Yellow silt loam	8.7
		56–78	7. Gray silt loam	8.7
			Holdrege silt loam	
11	Franklin Co., Nebr.	0–6	1. Very dark grayish brown silt loam	7.5
		6–18	2. Very dark grayish brown granular silt loam	7.1
		18–26	3. Compact zone. Dark grayish brown silt loam	7.2
		26–40	4. Lime zone. Very light grayish brown silt loam	8.3
		40–121	5. Lime zone. Very light grayish brown silt loam	8.5
		121–168	6. Loess-like silt loam, very light grayish brown loess	8.5

TABLE 2—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Holdrege silt loam—Continued	
12	Buffalo Co., Nebr.	0–6	1. Very dark brown silt loam	6.7
		6–14	2. Dark brown heavy silt loam	6.5
		14–26	3. Light brown heavy silt loam	6.8
		26–32	4. Compact zone. Dark grayish brown heavy silt loam	6.9
		32–60	5. Lime zone. Very light grayish brown silt loam with soft lime lumps	8.1
13	Phelps Co., Nebr.	0–5	1. Very dark grayish brown silt loam	6.4
		5–17	2. Dark brown silt loam	5.9
		17–36	3. Compact zone. Dark grayish brown heavy silt loam	7.3
		36–54	4. Lime zone. Very light grayish brown silt loam with soft lime lumps	8.5
		54–96	5. Very light gray silty loess	8.6
14	Furnas Co., Nebr.	0–½	1. Dark grayish brown silt loam mulch	6.4
		½–2	2. Very dark grayish brown faintly laminated silt loam	6.4
		2–16	3. Very dark brown friable silt loam	6.6
		16–26	4. Compact zone. Dark grayish brown silty clay loam	7.1
		26–40	5. Lime zone. Very light grayish brown friable silt loam	8.4
		40–55	6. Lime zone. Grayish brown silt loam	8.5
		55–84	7. Very light gray silt loess	8.4

CHESTNUT SOILS

Four soils series were selected as representative of the chestnut soils (fig. 1) (23, 38, 44, 70). They are the Morton (15, 16, 17, 18, 19, 22), Scobey, Williams (15, 16, 17, 18, 19), and Rosebud (24, 42, 72).

The pH values of the chestnut soils are just a trifle higher than those of the chernozems (tables 2 and 3). They range from 6.3 to 8.6 for the horizons above the lime zones and from 7.7 to 9.0 for the lime zones. These findings agree very closely with the results obtained by other investigators (6, 50, 65). Of the 13 profiles studied, 3 have slightly acid horizons above the lime zone, 8 have upper horizons that are neutral, and 2 have horizons that are alkaline (tables 1 and 3). In every profile the lime zone is more alkaline than any horizon above it, although in 6 profiles the horizon just above the lime zone has almost the same pH as that layer. Of the 13 profiles, 11 have horizons more alkaline than 8.5. In all but one case these strongly alkaline layers occur in the lime zone or below. In the one exception the compact zone is also highly alkaline. In 6 profiles the compact zone has virtually the same pH as the surface horizon, in 5 the compact
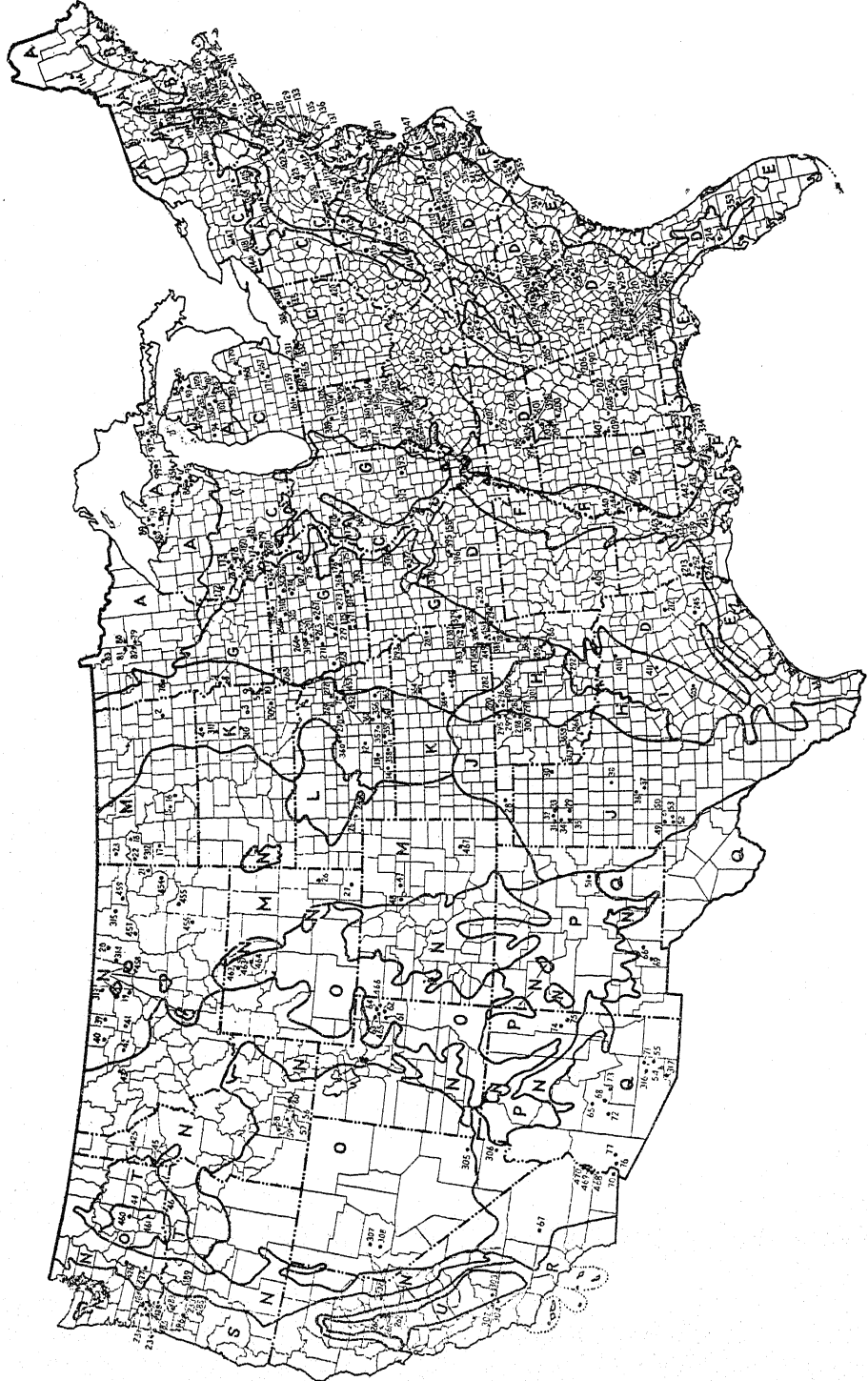
Fig. 1

zone is intermediate in pH between the surface and the lime zone, and in 1 the compact layer has virtually the same pH as the lime zone. There is very little difference between the range in pH values of the profiles of the four chestnut soils studied. The Morton averages the lowest, and the Scobey and Williams average the highest, doubtless largely because of the differences in lime content of the parent materials of these soils. The Scobey profiles are slightly more alkaline in their upper horizons than the Williams profiles. This is what one would expect, as the former has developed under a slightly lighter rainfall than the latter (15, 16, 17, 18, 19, 22).

FIG. 1. BROAD ASSOCIATIONS OF THE GREAT SOIL GROUPS

A—Podzols with associated ground water podzols, lithosols, bog and half-bog soils.

B—Brown podzolic soils, lithosols, bog and half-bog soils.

C—Gray-brown podzolic soils, with planosols, prairie soils, Wiesenböden, bog, half-bog, and alluvial soils.

D—Red and yellow podzolic soils, with lithosols, planosols, alluvial soils, rendzinas, Wiesenböden, and some prairie and reddish prairie soils in the northwestern part.

E—Half-bog soils, with ground water podzols predominating in the southeastern portion and included areas of red and yellow podzolic soils, bog soils, and alluvial soils.

F—Alluvial soils, with bog and half-bog soils and some red and yellow podzolic soils.

G—Prairie soils, with many areas of planosols, and some Wiesenböden and gray-brown podzolic soils. There are also areas of alluvial soils, reddish prairie soils, lithosols, and chernozems.

H—Reddish prairie soils and planosols with many areas of red and yellow podzolic soils, also some rendzinas, prairie soils reddish chestnut soils, and lithosols.

I—Rendzinas, with included areas of red and yellow podzolic soils, prairie, reddish prairie, reddish chestnut, Wiesenböden, and half-bog soils.

J—Reddish chestnut soils, with reddish brown soils predominant in the western portion and some lithosols, planosols, and reddish prairie soils.

K—Chernozems, with some planosols, Wiesenböden, alluvial, and prairie soils.

L—Dry sands.

M—Chestnut soils in the eastern part and brown soils in the western part with lithosols, dry sands, alluvial soils, and some chernozems.

N—Mountainous areas of brown and gray-brown podzolic soils, some Alpine meadow, podzols, much lithosol, and irregular areas of brown, chestnut and prairie soils.

O—Sierozem and desert soils, with much lithosol, and considerable solonchak, solonetz, brown, chestnut and alluvial soils.

P—Brown and chestnut soils, with much lithosol, and some sierozem, desert and red desert soils.

Q—Red desert, reddish brown, and noncalcic brown soils, with much lithosol and some alluvial soils and solonchak.

R—Mountain and valley areas of noncalcic brown soils, rendzinas, lithosols, planosols, alluvial soils, prairie, reddish chestnut, chernozem, half-bog and bog soils.

S—Mountain and valley areas of red, yellow, brown and gray-brown podzolic soils and lithosols, with some prairie soils, planosols, alluvial soils, half-bog and bog soils.

T—Chestnut, brown, prairie, and chernozem soils with some lithosols.

U—Central valley of California, dominantly alluvial soils and planosols, with included areas of solonchak, solonetz, rendzinas, noncalcic brown, reddish-brown, reddish chestnut, desert, bog, half-bog, chernozem, and prairie soils.

Each number represents a profile.

TABLE 3
Chestnut soils

PRO-FILE NUM-BER	LOCATION	DEPTH inches	HORIZON DESCRIPTION	pH
			Morton silt loam	
15	Morton Co., N. Dak.	0–1½	1. Dark gray-brown silt loam, crumb structure	6.8
		1½–6	2. Compact zone. Dark grayish brown silt loam prismatic structure	6.5
		6–12	3. Compact zone. Dark brown to brown heavy silt loam, good prismatic structure	6.7
		12–17	4. Compact zone. Olive-drab soft friable loam to silt loam, fair prismatic structure	7.1
		17–20	5. Lime zone. Olive-drab clay loam, friable, with lime flecks and considerable gravel	8.1
		20–38	6. Lime zone. Gray clay loam with a great abundance of lime flecks	8.4
		38–50	7. Lime zone. Cream-gray clay loam with fewer lime flecks, some limonite yellow specks and streaks	8.7
16	Morton Co., N. Dak.	0–6	1. Dark gray-brown friable silt loam	6.4
		6–14	2. Compact zone. Reddish brown heavy silt loam, good prismatic structure	6.3
		14–18	3. Olive-brown loam, breaks to large nut fragments and crumbs more easily	6.7
		18–30	4. Lime zone. Dark brown gravelly loam with lime flecks	8.1
			Morton loam	
17	Billings Co., N. Dak.	0–¾	1. Brownish gray powdery loam	7.2
		¾–2	2. Dark grayish brown loam in large subangular chunks but friable	7.2
		2–6	3. Dark brown silt loam or loam, cloddy	7.1
		6–12	4. Compact zone. Grayish brown silt loam with a tendency toward prismatic structure	7.1
		12–18	5. Compact zone. Brownish yellow silt loam with a tendency toward prismatic structure	7.6
		18–39	6. Lime zone. Light gray mottled with yellowish gray silt loam, heavily impregnated with lime	8.5
		39–48	7. Lime zone. Light gray-brown sand and gravel with an uneven coat of lime on their surfaces	8.8
18	McKenzie Co., N. Dak.	0–1¼	1. Dark grayish brown loam, friable crumb structure	7.2
		1¼–3½	2. Dark grayish brown loam, slightly lighter in color, friable crumb structure	6.8
		3½–9	3. Dark grayish brown loam, slightly lighter in color, friable crumb structure	6.8
		9–18	4. Compact zone. Grayish brown loam, prismatic structure	7.0
		18–25	5. Light grayish brown friable loam	7.5
		25–31	6. Lime zone. Light grayish brown calcareous loam, a few lime flecks	7.7
		31–36	7. Lime zone. Grayish white loam, numerous lime flecks	8.6

TABLE 3—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Scobey clay loam	
19	Milk River Valley Area, Mont.	0–2	1. Brown friable loam, fine granular structure	7.4
		2–8	2. Compact zone. Dull brown dense cloddy clay, faintly prismatic structure	7.1
		8–19	3. Lime zone. Grayish brown tough heavy clay loam distinctly calcareous	8.6
		19–35	4. Lime zone. Light grayish brown clay loam mottled with white and rusty brown calcareous	7.8
		35–45	5. Light grayish brown compact loamy material somewhat stratified and mottled with rusty brown and flecked with white salt accumulations	7.8
			Scobey loam	
20	Northern Plains of Montana	0–2	1. Dark brown loose friable mulch	7.1
		2–6	2. Dark brown more compact yet friable crumb structure, coarse loam	7.1
		6–10	3. Dark brown more compact friable loam to silt loam, crumb structure	8.2
		10–16	4. Compact zone. Hard compact dull dark brown heavy tight loam, prismatic structure	8.6
		16–30	5. Lime zone. Dull light brown heavy loam to silt loam, tight and compact, calcareous	8.9
		30–36	6. Mottled light yellowish brown and grayish brown compact crumb structure loam to silt loam	8.3
			Williams loam	
21	Lower Yellowstone Area, Mont.	0–6	1. Dark brown loam, laminated at surface, blocky below	7.7
		6–16	2. Compact zone, brown loam with fine gravel stone scattered through, prismatic, granular structure	7.5
		16–30	3. Lime zone. Friable light yellowish gray silt loam streaked and spotted with lime	8.7
		30–45	4. Lime zone. Compact olive-brown silt loam spotted with lime	9.0
			Williams clay loam	
22	McKenzie Co., N. Dak.	0–1	1. Dark grayish brown friable clay loam, crumb structure	7.4
		1–3	2. Dark grayish brown friable clay loam, crumb structure	6.7
		3–8	3. Dark grayish brown compact silt loam but friable, crumb structure	7.2

TABLE 3—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Williams clay loam—Continued	
22	McKenzie Co., N. Dak.—*Cont.*	8–12	4. Compact zone. Dark grayish brown clay loam, prismatic structure	8.3
		12–18	5. Lime zone. Grayish brown friable clay loam with whitish lime flecks	8.5
		18–38	6. Lime zone. Light gray friable clay loam with more numerous white lime flecks	8.9
		38–49—	7. Light grayish brown friable calcareous clay loam	9.3
			Williams loam	
23	Williams Co., N. Dak.	0–1½	1. Dark grayish brown light loam, fine soft crumb structure	6.9
		1½–5	2. Dark brown loam, soft crumb structure also faintly platy	7.0
		5–13	3. Compact zone. Reddish brown sandy clay, prismatic structure	7.2
		13–20	4. Compact zone. Grayish brown clay loam, prismatic structure, a little less friable	8.0
		20–24	5. Lime zone. Light olive-drab sandy clay till strongly mottled with white lime spots; compact but friable	8.2
		24–	6. Lime zone. Light olive-drab sandy clay till strongly mottled with white lime spots; loose and friable	8.8
			Rosebud very fine sandy loam	
24	Lincoln Co., Nebr.	0–1	1. Light gray very fine sandy loam, single grain	6.4
		1–8	2. Compact zone. Reddish brown very fine sandy loam, breaks into sharp angular clods when dry—prismatic structure	6.3
		8–14	3. Dark grayish brown silt loam, loose to friable	7.2
		14–24	4. Lime zone. Light yellowish brown silt loam, numerous lime streaks and splotches	7.7
		24–36	5. Lime zone. Light yellowish brown silt loam, fewer lime streaks and splotches	8.7
		36–90	6. Yellow fine sand	8.5
25	Keith Co., Nebr.	0–2	1. Light brown very fine sandy loam	7.3
		2–7	2. Dark brown very fine sandy loam	6.8
		7–15	3. Compact zone. Brown very fine sandy loam faintly granular, somewhat compact, prismatic structure	7.0
		15–28	4. Compact zone. Brown very fine sandy loam, prismatic structure to faintly granular	7.7
		28–36	5. Lime zone. Light yellow silt loam	8.3
		36–48	6. Light yellow silt loam containing some gravel	8.4
		48–72	7. Light yellow silt loam	8.4

TABLE 3—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Rosebud very fine sandy loam—Continued	
26	Fort Laramie Area, Wyo.	0–8	1. Brown friable very sandy loam	7.4
		8–30	2. Lime zone. Yellowish gray silty clay loam	8.3
		30–48	3. Grayish silt and very fine sand	9.0
			Rosebud silt loam	
27	Laramie Co., Wyo.	0–3½	1. Brown laminated silt loam	7.1
		3½–12	2. Compact zone. Dark brown heavy silt loam, slightly prismatic structure	8.1
		12–20	3. Compact zone. Brown heavy silt loam, prismatic structure	8.0
		20–33	4. Lime zone. Light yellow friable silt loam with numerous lime spots	8.5
		33–39	5. Light yellow silt loam	8.8

REDDISH CHESTNUT SOILS

Three series were selected as representative of the reddish chestnut soils (fig. 1) (44, 70).   They are the Richfield (2, 21, 63), Pullman (62, 63), and Abilene (11, 64).

The pH values of the horizons above the lime zones of these profiles range from 6.5 to 8.2 and those of their lime zones from 7.5 to 8.5.   These results are similar to those of the chestnut profiles (tables 3 and 4).   Of the 11 profiles studied, 1 has a slightly acid horizon above the lime zone, 5 have upper horizons that are neutral, and 5 are alkaline from the surface down (tables 1 and 4).   In only 4 profiles is the lime zone more alkaline than any horizon above it.   None of the profiles have horizons more alkaline than 8.5.   In 4 profiles the compact zone is intermediate in pH between the surface and the lime zone, in 3 the compact layer has virtually the same pH as the lime zone, in 1 the pH of the compact zone is virtually the same as that of the surface, in 1 the compact zone has the lowest pH of any horizon in the profile, and 1 profile has virtually the same pH throughout.

The pH values of the three Richfield profiles range from 7.1 to 8.5.   Two profiles have some horizons above the lime zone that are neutral, and one profile is mildly alkaline in its upper layers.

The pH values of the 5 Pullman profiles vary from 6.5 to 8.5.   This variation agrees with the data reported by Brown and Byers on a Pullman profile (6).   One profile has a slightly acid horizon above the lime zone, 3 have neutral horizons in the upper layers and 1 is mildly alkaline above the lime zone.   Thus these profiles average the lowest in pH in the upper horizons of the reddish chestnut soils studied, although the differences are very slight (table 4).   In 2 profiles there are horizons above the lime zone that are just as alkaline as that horizon or more so.   In one profile the equally alkaline horizon is in the compact

TABLE 4
Reddish chestnut soils

PRO-FILE NUMBER	LOCATION	DEPTH inches	HORIZON DESCRIPTION	pH
		Richfield silt loam		
28	Texas County, Oklahoma Reconnaissance	0–4	1. Very dark reddish brown laminated silt loam	7.2
		4–12	2. Compact zone. Very dark reddish brown clay loam with compact prismatic structure	7.7
		12–20	3. Lime zone. Dark reddish brown clay loam, very compact block structure, white lime spots	8.5
		20–28	4. Lime zone. Reddish brown clay loam or clay with spots of lime, very hard compact cubical clods	8.5
		28–32	5. Lime zone. Light grayish brown with numerous lime spots, clay to clay loam	8.5
		32–42	6. Lime zone. Brown clay loam with fewer lime spots, blocky structure	8.5
		42–52	7. Light brown clay loam	8.5
		52–72	8. Friable light brown to slightly reddish brown clay to clay loam	8.5 8.5
		Richfield silty clay loam		
29	Randall County, Texas	0–5	1. Very dark reddish brown, granular silty clay loam	8.1
		5–18	2. Compact zone. Nearly black granular silty clay loam, roughly prismatic structure	7.6
		18–30	3. Lime zone. Light grayish brown clay with numerous lime concretions	8.0
		30–42	4. Lime zone. Light grayish brown friable clay with soft lime lumps	8.5
30	Wheeler County, Texas	0–1	1. Very dark reddish brown, very fine sandy loam, single grain structure	7.1
		1–8	2. Compact zone. Nearly black granular clay loam, roughly prismatic structure	7.0
		8–18	3. Compact zone. Reddish brown to black heavy clay loam, nut-like structure	7.1
		18–27	4. Compact zone. Reddish brown stiff clay loam, roughly nut-like structure	7.5
		27–48	5. Lime zone. Reddish brown to brown plastic clay with numerous soft lime spots	8.3
		Pullman silty clay loam		
31	Potter County, Texas	0–5	1. Dark reddish brown silty clay loam	6.7
		5–26	2. Compact zone. Dark chocolate-brown stiff clay	7.7
		26–48	3. Compact zone. Dark reddish brown stiff clay	8.2
		48–66	4. Compact zone. Yellowish red stiff clay	8.0
		66–85	5. Lime zone. Dull reddish yellow friable clay containing many soft white lime lumps	8.0
		85–102	6. Parent material. Pinkish buff calcareous clay	8.3

TABLE 4—*Continued*

PRO-FILE NUMBER	LOCATION	DEPTH inches	HORIZON DESCRIPTION	pH
			Pullman silty clay loam—Continued	
32	Potter County, Texas	0–5	1. Very dark reddish brown silty clay loam	6.6
		5–26	2. Compact zone. Dark chocolate-brown clay	7.3
		26–42	3. Dark brown calcareous clay	8.2
		42–60	4. Yellowish red calcareous clay	7.9
		60–84	5. Lime zone. Friable buff clay with soft lime lumps	7.9
		84–134	6. Parent material. Pale reddish buff calcareous clay	8.0
33	Potter County, Texas	0–4	1. Very dark reddish brown granular to platy silty clay loam	6.6
		4–14	2. Compact zone. Nearly black plastic granular and finely cloddy clay	6.5
		14–21	3. Compact zone. Reddish brown granular fine cloddy plastic clay	7.3
		21–32	4. Compact zone. Reddish brown calcareous plastic cloddy clay	8.0
		32–48	5. Dull yellowish red calcareous clay	8.0
		48–84	6. Lime zone. Soft yellowish white almost pure lime	8.3
		84–108	7. Pale reddish buff calcareous clay with some lime concretions	8.2
34	Randall County, Texas	0–5	1. Friable dark reddish brown silty clay loam	7.6
		5–24	2. Compact zone. Very dark chocolate stiff heavy clay loam	8.0
		24–36	3. Lime zone. Reddish brown stiff clay containing a few soft lime concretions	8.3
		36–50	4. Lime zone. Dull reddish yellow clay containing a few soft lime concretions	8.5
		50–60	5. Lime zone. Friable buff clay containing 50 per cent or more white soft lumps of lime	8.5
		60–144+	6. Parent material. Pinkish buff calcareous clay with some soft lime lumps	8.5
35	Randall County, Texas	0–2	1. Dark reddish brown silty clay loam	7.2
		2–12	2. Compact zone. Dark reddish brown stiff heavy clay	6.6
		12–24	3. Compact zone. Dark reddish brown stiff heavy clay, slightly lighter in color	7.6
		24–40	4. Lime zone. Dark reddish brown clay containing a few semihard lime concretions, slightly lighter in color	7.5
		40–52	5. Lime zone—brown calcareous clay	7.8
		52–60	6. Lime zone, yellowish red calcareous clay, lime concretions increasing	7.8
		60–78	7. Lime zone. Friable buff clay 50 per cent or more soft lime lumps	8.0
		78–90+	8. Parent material. Pinkish buff calcareous friable clay with a few soft lime lumps	7.9

TABLE 4—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Abilene loam	
36	Scurry County, Texas	0–7	1. Dark reddish brown friable loam	7.8
		7–18	2. Compact zone. Very dark reddish brown stiff clay	8.0
		18–32	3. Lime zone. Whitish and soft, nearly pure lime	8.1
		32–48	4. Pinkish, whitish and salmon friable clay, crystals of gypsum abundant	8.3
			Abilene clay loam	
37	Scurry County, Texas	0–1	1. Dark grayish brown with a red tint, silt loam, arranged in thin fragile plates	7.8
		1–16	2. Dark reddish brown granular clay loam	7.7
		16–30	3. Compact zone. Dark reddish brown rather compact clay with a prismatic structure	8.0
		30–42	4. Lime zone. Light reddish brown compact clay containing a few lime spots	8.0
		42–66	5. Lime zone. Yellowish white mixture of white rounded lime lumps and friable light brown clay	8.0
		66–144	6. Parent material. Pale reddish brown friable firm cloddy clay containing a few lumps of lime	8.0
			Abilene silty clay loam	
38	Dickens County, Texas	0–4	1. Dark reddish brown silty clay loam	7.7
		4–42	2. Compact zone. Very dark reddish brown stiff clay	7.8
		42–60	3. Lime zone. Whitish and soft, nearly all lime	7.6
		60–72	4. Pinkish whitish and salmon friable clay, gypsum crystals abundant	7.5

layer, whereas in the other profile the more alkaline horizon is the transitional layer between the compact and the lime zones.

The pH values of the three Abilene profiles range from 7.5 to 8.3. These pH values have the narrowest range of any of the reddish chestnut profiles studied. In all these soils the horizons above the lime zone are mildly alkaline (tables 1 and 4). These profiles have the highest pH in the upper horizons of any of the reddish chestnut soils studied.

BROWN SOILS

Three series were selected as representative of the brown soils (fig. 1) (70). They are the Joplin (22), Ritzville (29, 48, 56, 71, 79), and Fort Collins (26).

The pH values of the horizons above the lime zones of these profiles vary from 6.1 to 8.1, and those of the lime zones from 7.8 to 9.4 (table 5). These are considerably higher, especially in the upper horizons, than the data reported by

TABLE 5

Brown soils

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Joplin fine sandy loam	
39	Northern Plains of Montana	0–1	1. Grayish brown loose granular to single-grain sand mulch	6.2
		1–6	2. Compact zone. Medium brown fine sandy loam, crumbly prismatic structure	7.1
		6–11	3. Compact zone. Light brown fine sandy loam, friable prismatic structure	7.7
		11–14	4. Compact zone. Grayish brown fine sand to silt, compact prismatic structure	8.1
		14–23	5. Lime zone. Lime well distributed, gray silt, compact prismatic structure	8.4
		23–32	6. Lime zone. Grayish brown silt loam, patches or streaks of lime, compact irregular clods	8.4
		32–45	7. Alkali zone. Dull brown silty clay granular, lime and alkali in patches, gravel, shale and sandstone present	8.1
		45–48	8. Light brown compact gravelly fine sand to silt layer	8.0
		48–59	9. Light brown compact stratified silt loam, free from gravel	7.7
		59–65	10. Reddish brown compact gritty silty clay, stratified	7.8
			Joplin loam	
40	Northern Plains of Montana	0–2	1. Light brown loose loam mulch	6.3
		2–3	2. Brown more compact coarse silt loam, crumbles easily	6.4
		3–8	3. Compact zone. Slightly darker brown compact heavy silt loam, blocky and prismatic structure	6.8
		8–10	4. Compact zone. Grayish brown compact silt loam, prismatic structure but more friable	7.8
		10–20	5. Lime zone. Brownish gray silt loam to loam with fine light gray specks of lime, slightly blocky but friable	8.1
		20–34	6. Lime zone. Light grayish brown silt loam and very fine sandy loam with large white spots of lime, very friable	7.9
		34–46	7. Light yellowish brown very fine sandy loam	8.1
			Joplin stony loam	
41	Northern Plains of Montana	0–2	1. Loose gray sandy mulch	7.9
		2–6	2. Reddish brown gravelly loam	7.6
		6–9	3. Light reddish brown gravelly loam	7.8
		9–24	4. Compact zone. Compact gray gravelly loam, prismatic structure	8.0

TABLE 5—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	HORIZON DESCRIPTION	pH
			Joplin stony loam—Continued	
41	Northern Plains of Montana	24–38	5. Lime zone. Rather loose yellow loam and gravel with numerous white lime spots	8.1
		38–50	6. Lime zone. Compact dull gray sand and gravel, lime well distributed	8.1
		50–62	7. Loose gray sand, underlain by heavier stratified material	8.1
			Joplin loam	
42	Northern Plains of Montana	0–3	1. Grayish brown loam mulch, slightly laminated	7.2
		3–11	2. Compact zone. Yellowish brown sandy loam, slightly compact, prismatic structure breaking into irregular cubical blocks	6.9
		11–14	3. Compact zone. Brownish yellow, prismatic structure	7.5
		14–22	4. Lime zone. Light grayish brown fine sandy loam with white splotches of lime, slightly prismatic structure	8.0
		22–26	5. Lime zone. Light grayish brown structureless fine sandy loam, lime well distributed	8.1
		26–42	6. Dark drab-brown silty clay drift, compact, flaky, contains bits of shale and sandstone	8.4
		42–60	7. Dull brown drift mixture, some gravel and rock fragments	8.0
			Joplin silt loam	
43	Northern Plains of Montana	0–2	1. Light brown silt loam mulch	6.1
		2–7	2. Compact zone. Brown hard compact heavy loam, prismatic structure	7.3
		7–10	3. Lime zone. Grayish brown hard compact heavy loam, prismatic structure, fine specks of lime	7.8
		10–20	4. Lime zone. Light gray and light brown speckled blotched heavy silt loam, lime well distributed, prismatic structure	8.2
		20–30	5. Lime zone. Less gray and more brown heavy silt loam, lime well distributed	8.0
		30–40	6. Light dull brown with dirty gray, hard, compact, gritty silt loam	8.0
			Ritzville silt loam	
44	Franklin Co., Wash.	0–12	1. Light grayish brown friable silt loam	7.0
		12–36	2. Compact zone. Light brown cloddy heavy silt loam to silty clay loam, prismatic structure	8.0
		36–72	3. Lime zone. Grayish brown to gray heavy silt loam, slightly cemented	8.9

TABLE 5—*Continued*

PROFILE NUMBER	LOCATION	DEPTH inches	HORIZON DESCRIPTION	pH
			Ritzville silt loam—Continued	
45	Nez Perce Co., Idaho	0–2½	1. Light grayish brown friable silt loam	7.3
		2½–8	2. Compact zone. Light brown heavy silt loam, prismatic structure	7.1
		8–19	3. Compact zone. Light brown cloddy silty clay loam, prismatic structure	7.0
		19–33	4. Lime zone. Grayish brown firm but friable silt loam with numerous white lime spots and streaks	8.2
46	Umatilla Co., Oreg.	0–8	1. Light brown friable silt loam to loam	6.7
		8–19	2. Compact zone. Light brown heavy loam, prismatic structure	6.7
		19–33	3. Compact zone. Light brown firm but friable silt loam, prismatic structure	7.4
		33–46	4. Lime zone. Grayish brown firm but friable silt loam, calcareous	8.9
		46–66	5. Lime zone. Grayish brown firm but friable silt loam, calcareous	9.4
		66–86	6. Lime zone. Grayish brown to gray heavy silt loam, slightly cemented with lime	9.1
			Fort Collins loam	
47	Brighton Area, Colo.	0–15	1. Brown friable, loam with few waterworn gravels	8.0
		15–36	2. Compact zone. Brown clay, prismatic structure	7.3
		36–60	3. Lime zone. Olive-brown with gray mottlings and lime streaks, heavy structureless zone	8.6
			Fort Collins clay loam	
48	Brighton Area, Colo.	0–2	1. Grayish brown mellow fine or very fine sandy loam, slightly laminated	7.3
		2–8	2. Gray brown to brown heavy loam	6.2
		8–22	3. Compact zone. Brown to dark brown compact clay, prismatic structure	7.6
		22–38	4. Lime zone. Grayish brown compact clay of prismatic structure, mottlings of lime	8.4
		38–54	5. Lime zone. Gray clay loam very high in lime, massive structure	9.1
		54–96	6. Lime zone. Pinkish gray heavy clay loam to clay, lime in seams downward and in pockets, prismatic structure	9.1
		96–120	7. Parent material. Light brown highly calcareous fine sandy loam	9.1

Thorp and Hou (65, 66) on Chinese soils and those of Wyatt and Newton on western Canadian soils (75).

Of the 10 profiles studied, 4 have some horizons above the lime zone that are slightly acid, 5 are neutral in some of the upper horizons, and 1 profile is mildly alkaline above the lime zone (tables 1 and 5). Thus the pH values of these brown soils are virtually the same as those of the other grassland pedocals; namely, the chernozems and the chestnut and reddish chestnut soils (tables 2 to 5). They actually average a trifle lower in pH values in the upper layers than do the chestnut soils (Tables 3 and 5). This seems very strange, as one would expect the upper horizons, at least, to become increasingly more alkaline as the rainfall decreases. The brown soils selected are coarser textured on the average, especially in their upper horizons, than the chestnut profiles selected (tables 3 and 5). This would permit a more rapid percolation of water through the upper layers and a consequent leaching out of the bases. This undoubtedly accounts in part at least, for their relatively lower pH values.

As in the chestnut soils, the pH values of the lime zones tend to be higher than those of the layers above, although in 3 profiles the lowest horizon in the compact zone has virtually the same pH values as the lime zone. Of the 10 profiles studied, 4 have horizons more alkaline than 8.5. In every case these strongly alkaline layers occur in the lime zone or below. In 6 profiles the pH of the compact layer is intermediate between that of the surface and that of the lime zone, in 2 profiles it has the same pH as the surface, in 1 it has virtually the same pH as the lime zone, and in 1 it has the lowest pH in the profile.

The pH values of the 5 Jopkin profiles range from 6.1 to 8.4. Three profiles have some horizons above the lime zone that are slightly acid, one has neutral horizons in the upper layers, and one profile is mildly alkaline above the lime zone. These profiles average the least alkaline of the brown soils studied.

The 3 profiles of the Ritzville series range in pH from 6.7 to 9.4. These are the most alkaline profile reactions of this group, especially in the upper horizons. All of the profiles are neutral to mildly alkaline above the lime zone (tables 1, 5). The slightly cemented horizon in the lime zone of one of the profiles happens to be the deepest layer collected in that profile, and it has the highest pH value in that profile.

The pH values of the 2 Fort Collins profiles vary from 6l2 to 9.1. One profile has a slightly acid horizon above the lime zone, whereas the other one is neutral to mildly alkaline in the upper layers. The fact that the finer textured profile has lower pH values in the horizons above the lime zone than the coarser textured one appears to contradict the hypothesis earlier advanced for the relatively low pH values of the upper horizons of the brown soils as compared with the more humid pedocals. Obviously this difference in pH values must be due to some cause other than texture.

REDDISH BROWN SOILS

Three series were selected as representative of the reddish brown soils (fig. 1) (70). They are the Springer (60, 61), Tivoli (80,) and White House (80). The

TABLE 6

Reddish brown soils

PRO-FILE NUM-BER	LOCATION	DEPTH inches	HORIZON DESCRIPTION	pH
			Springer loam	
49	Midland Co., Tex.	0–1½	1. Platy, chocolate-brown loam	6.7
		1½–6	2. Reddish brown massive loam	6.8
		6–36	3. Compact zone. Red massive clay loam on hard CaCO₃	7.3
			Springer fine sandy loam	
50	Midland Co., Tex.	0–6	1. Reddish brown fine sandy loam	7.4
		6–14	2. Light reddish brown fine sandy loam	6.9
		14–48	3. Compact brownish red fine sandy clay loam	7.7
		48–60	4. Lime zone. Brownish red fine sandy clay, some lime streaks	8.0
		60–72	5. Lime zone. Brownish red fine sandy clay, more lime streaks	8.4
		72–84	6. Lime zone. Yellow fine sandy limey clay, occasional lime concretions on hard CaCO₃	8.4
51	Ft. Sumner Area, N. Mex.	0–1½	1. Reddish brown fine sandy loam	6.4
		1½–10	2. Reddish brown fine sandy loam slightly lighter in color	7.0
		10–18	3. Reddish brown fine sandy loam slightly lighter in color	7.9
		18–28	4. Lime zone. Light reddish brown fine sandy loam, some lime streaks	7.9
		28–32	5. Lime zone. Pinkish caliche	8.1
52	Midland Co., Tex.	0–5	1. Dark reddish brown heavy fine sandy loam	7.7
		5–16	2. Compact zone. Brown to dark reddish brown clay loam	7.7
		16–36	3. Lime zone. Calcareous light brownish red clay loam on soft CaCO₃	7.9
			Tivoli fine sand	
53	Midland Co., Tex.	0–95	1. Buff to grayish yellow fine sand	6.6
		95–104	2. Reddish yellow fine sandy loam	5.8
		104–124	3. Compact zone. Reddish yellow fine sandy clay loam to fine sandy clay	6.7
		124–168+	4. Lime zone. Grayish yellow loam to fine sandy loam, highly calcareous with some soft lime concretions	8.1
			White House gravelly sandy loam	
54	Tucson Area, Ariz.	0–1½	1. Light brownish red loose gravelly sandy loam	5.8
		1½–10	2. Dark reddish brown friable sandy loam, granular, contains much organic matter	6.2

TABLE 6—*Continued*

PRO-FILE NUMBER	LOCATION	DEPTH inches	HORIZON DESCRIPTION	pH
			White House gravelly sandy loam—Continued	
54	Tucson Area, Ariz.	10–28	3. Compact zone. Deep dark red clay, dense cloddy structure, contains cobbles and boulders	6.7
		28–45	4. Compact zone. Brownish red tough heavy clay containing much disintegrating rhyolite, trachyte, granite, syenite, and diorite	7.0
		45–60	5. Lime zone. Highly calcareous disintegrating rock with tongues of the above tough red clay	8.3
			White House coarse sandy loam	
55	Tucson Area, Ariz.	0–1½	1. Light brownish red loose loamy coarse sand	6.2
		1½–7	2. Dark reddish brown friable coarse sandy loam, probably contains considerable organic matter	6.8
		7–12	3. Compact zone. Dark brownish red gritty clay loam, rather compact, but softer than horizon below	6.8
		12–30	4. Compact zone. Dark red compact cloddy gritty clay, indistinct columnar structure	7.2
		30–50	5. Lime zone. Brownish red clay loam or clay, contains disintegrating gravel and stone, distinctly calcareous	8.3

pH values of the horizons above the lime zones range from 5.8 to 7.9 and those of the lime zones from 7.9 to 8.4. Of the 7 profiles studied, 3 have neutral horizons above the lime zone, 1 is mildly alkaline throughout the horizons collected, 1 has a slightly acid layer above the lime layer, and 2 are medium-acid in the upper horizons. These soils average the most acid of the grassland pedicals (tables 2 to 6). This is doubtless due to the sandy nature of these profiles. A further indication of this is the fact that the sandiest one of the Springer profiles has the lowest pH in one of its upper horizons and the more gravelly of the White House profiles has the most acid horizons of that series in its surface layer.

SIEROZEM SOILS

The Portneuf series (49, 76, 79) was selected as representative of the sierozem soils (fig. 1) (70). The pH values of the horizons above the lime zones vary from 7.3 to 8.5 and those of the lime zones from 8.3 to 8.9 (table 7). One profile has a horizon above the lime zone that is neutral, 3 profiles have mildly alkaline horizons in the upper layers, and 1 profile is strongly alkaline throughout the profile. Of the Pedocals so far studied, these soils have the highest pH values in horizons above the lime zone (tables 2 to 7). Three profiles have horizons more alkaline than pH 8.5. In every case this high alkalinity occurs in the lime zone and the parent material. In 3 of the 4 profiles from which samples of the surface horizons were available, the compact zone has the lowest pH in the profile,

TABLE 7
Sierozems

PRO-FILE NUMBER	LOCATION	DEPTH *inches*	DESCRIPTION OF HORIZON	pH
			Portneuf silt loam	
56	Minidoka Area, Idaho	0–2	1. Light brown friable silt loam to very fine sandy loam	7.4
		3–10	2. Compact zone. Brown silt loam, slightly compact prismatic structure	7.3
		11–26	3. Lime zone. Yellowish white nodular silt loam, very high in lime content	8.4
		27–72	4. Parent material. Brown very fine sandy loam	8.4
57	Jerome Area, Idaho	0–2½	1. Light grayish brown silt loam, friable and smooth, slightly platy	7.6
		2½–15	2. Compact zone. Light reddish brown heavy silt loam to silty clay loam, prismatic structure, plastic when wet	7.4
		15–25	3. Lime zone. Very light gray, silty clay loam, nodular to irregular nut-structure, compact but brittle, highly calcareous	8.8
		25–48	4. Lime zone. Very light gray silty clay loam, compact, brittle and hard, highly calcareous	8.9
		48–72	5. Parent material. Light yellowish gray very fine sandy loam, mellow and friable, highly calcareous	8.6
			Portneuf fine sandy loam	
58	Gooding Area, Idaho	0–8	1. Compact zone. Light brown slightly compact fine sandy loam, prismatic structure	8.5
		8–18	2. Lime zone. Rather compact fine sandy loam, highly calcareous and containing a few lime nodules	8.4
		18–31	3. Lime zone. Light gray compact very fine sandy loam, highly calcareous and softly cemented, nodular structure	8.6
		31–42	4. Lime zone. Light grayish brown friable fine sandy loam with a few fine nodules	8.6
		42–54	5. Lime zone. Gray and reddish brown mottled clay loam, softly cemented	8.6
59	Jerome Area, Idaho	0–8	1. Light brown mellow fine sandy loam	7.8
		8–20	2. Compact zone. Light reddish brown compact fine sandy loam, plastic, prismatic structure	7.7
		20–32	3. Lime zone. Light yellowish gray plastic very heavy silt loam, nut structure, lime, seamed	8.4
		32–72	4. Light yellowish brown fine sandy loam fairly compact but crumbling with pressure	8.5

TABLE 7—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	DESCRIPTION OF HORIZON	pH
		Portneuf very fine sandy loam		
60	Minidoka Area, Idaho	0–1	1. Light brown mellow very fine sandy loam	7.6
		1–6	2. Compact zone. Brown very slightly compact very fine sandy loam, prismatic structure	8.2
		6–18	3. Lime zone. Light brown friable very fine sandy loam, highly calcareous, containing a few soft lime nodules	8.3
		18–40	4. Lime zone. Very light brown to cream-colored silt loam, compact and nodular, very high in lime	8.8
		40–72	5. Parent material. Light yellowish brown very fine sandy loam, mostly loose and floury, but containing nodules of slightly cemented material	9.1

whereas in 1 profile the reaction of the compact zone is intermediate between that of the surface above and that of the lime zone below. In the profile that has the compact zone at the surface, the hydrogen-ion concentration of that layer is virtually the same as that of the lime zone. The two weakly cemented horizons in the lime zone have virtually the same pH values as the other horizons in that profile.

DESERT SOILS

The Mesa (32, 47) series was selected as representative of the desert soils (fig. 1) (70). The pH values of the 4 profiles studied range from 8.0 to 8.8 (table 8). One profile is midly to strongly alkaline above the lime zone, and the other three profiles are strongly alkaline throughout (tables 1 and 8). The horizons above the lime zones have virtually the same pH values as the lime zones. Thus the desert soils are considerably more alkaline than the sierozem soils in the upper layers, as one would expect. Three of the profiles have horizons above 8.5. In one profile these highly alkaline horizons occur throughout, whereas in two profiles they occur in the lower part of the lime zone or in the parent material. The cemented horizons of the lime zones appear to have pH values that are not essentially different from those of the other layers of the lime zones.

RED DESERT SOILS

Four series were selected as representative of the red desert soils (fig. 1) (70). They are the Mohave, Laveen, Moqui, and Superstition (26, 27, 56, 57, 59, 78). The pH values of the red desert soils studied range from 7.8 to 9.3 (table 9). These values are somewhat higher than those reported on this group of soils by Brown and Drosdoff (8) and by Lapham (41). Of the 13 profiles, 2 are mildly to strongly alkaline above the lime zones, whereas the rest are strongly alkaline in all of the upper layers (tables 1 and 9). Eleven profiles have more alkaline

TABLE 8

Desert soils

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	DESCRIPTION OF HORIZONS	pH
		Mesa clay loam		
61	Uinta River Valley Area, Utah	0–¼ ¼–3 3–36 36–48	1. Light brown clay loam, calcareous 2. Light brown heavy silt loam, calcareous 3. Lime zone. Light reddish brown heavy clay loam 4. Lime zone. Slightly pinkish very light gray clay, slightly cemented	8.5 8.4 8.2 8.6
62	Uinta River Valley Area, Utah	0–¼ ¼–2 2–24 24–42	1. Light grayish brown clay loam, calcareous 2. Light brown clay loam, calcareous 3. Lime zone. Light reddish brown clay loam, moderately compact, calcareous 4. Lime zone. Pinkish very light gray clay, slightly cemented	8.8 8.8 8.8 8.6
		Mesa sandy loam		
63	Uinta River Valley Area, Utah	0–½ ½–1½ 1½–18 18–60	1. Light reddish brown fine sandy loam, calcareous, containing small portions of lime crusts and large gravel 2. Light reddish brown fine sandy loam, calcareous, rounded cobbles and large gravel 3. Compact light reddish brown loam, moderately compact, calcareous, containing considerable quantities of water-worn cobbles and large gravel 4. Lime zone. Faint pinkish light gray clay in lime-coated gravels, slightly cemented	8.1 8.0 8.1 8.3
64	Uinta River Valley Area, Utah	0–¼ ¼–2 2–12 12–48	1. Light reddish brown fine sandy loam, slightly calcareous surface, scattering of small quartzite pebbles 2. Light reddish brown fine sandy loam, slightly calcareous 3. Lime zone. Light reddish brown fine sandy loam, moderately compact, containing a few small lime aggregates, scattered water-worn gravel 4. Lime zone. Lime-coated gravel embedded in faint pinkish very light gray clay, compact, slightly cemented	8.1 8.1 8.3 8.7

reactions than pH 8.5 in some of their horizons. Most of these highly alkaline horizons occur in the lime zone or in the parent material. Thus the pH values of the red desert soils are very similar to those of the desert soils, especially in the upper horizons, but are somewhat higher in their lower horizons (tables 8 and 9).

TABLE 9
Red desert soils

PRO-FILE NUM-BER	LOCATION	DEPTH inches	DESCRIPTION OF HORIZONS	pH
			Mohave sandy loam	
65	Buckeye-Beardsley Area, Ariz.	0–4	1. Faint reddish brown friable sandy loam, slightly calcareous	8.4
		4–18	2. Reddish brown friable sandy loam, slightly calcareous	8.4
		18–28	3. Compact zone. Grayish red, mottled, slightly compact heavy sandy loam or loam, highly calcareous	8.3
		28–50	4. Lime zone. Pinkish gray weakly cemented loam, very highly calcareous	8.4
		50–72	5. Brown coarse sand firm but friable, moderately calcareous	9.2
66	Denning Area, N. Mex.	0–1	1. Red loamy sand	8.2
		1–15	2. Compact zone. Red compact sandy loam	8.9
		15–25	3. Compact zone. Red heavy sandy loam	8.9
		25–60	4. Lime zone. Red loam with lime spots	8.2
67	Barstow Area, Calif.	0–3	1. Light grayish brown calcareous sandy loam	8.6
		3–10	2. Compact zone. Pinkish brown fairly compact sandy loam	8.8
		10–20	3. Compact zone. Light brown calcareous moderately compact sandy loam	9.3
		20–30	4. Lime zone. Light gray semicemented loam	8.8
		30–72	5. Rusty brown calcareous sand	9.1
			Mohave loam	
68	Salt River Valley Area, Ariz.	0–15	1. Brownish red to red gritty loam	8.0
		15–28	2. Compact zone. Brownish red to red silty clay loam, mildly calcareous	8.4
		28–42	3. Lime zone. Red moderately compact silty clay loam, moderately to highly calcareous	8.5
		42–72	4. Lime zone. Grayish brown to pinkish gray clay, weakly cemented	8.8
69	Denning Area, N. Mex.	0–1½	1. Reddish brown loam	8.5
		1½–12	2. Reddish brown loam, slightly lighter in color	8.0
		12–18	3. Compact zone. Reddish brown slightly compact loam	8.4
		18–60	4. Lime zone. Reddish brown loam with lime spots	8.5
			Mohave loamy coarse sand	
70	Yuma - Wellton Area, Ariz. —Calif.	0–10	1. Grayish brown loamy coarse rather loose sand	8.2
		10–20	2. Compact zone. Reddish brown coarse sandy loam, compact and cloddy, calcareous	8.2
		20–55	3. Lime zone. Reddish brown very compact sandy loam, containing many gray lime concretions	8.1
		55–72	4. Lime zone. Reddish brown rather compact loamy coarse sand, containing a few lime mottlings	8.7

TABLE 9—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH inches	DESCRIPTION OF HORIZONS	pH
			Laveen gravelly loam	
71	Santa Rita River Reservation, Ariz.	0–¾	1. Loose concentration of light grayish brown gravelly coarse sand and sandy loam, calcareous	8.3
		¾–14	2. Lime zone. Light grayish brown heavy sandy loam, firm but friable, highly calcareous, faintly veined with gray lime	8.3
		14–26	3. Lime zone. Light reddish brown friable gritty loam, veined and mottled with gray loam, containing considerable gravel	8.2
		26–44	4. Lime zone. Mottled light gray and pinkish brown gritty loam having rather compact, softly cemented, and nodular structure	8.1
		44–60	5. Lime zone. Rather firmly cemented material coming up in large chunks and containing considerable amounts of gravel and cobbles	8.3
		60–72	6. Lime zone. Light gray gravelly sand thoroughly impregnated with lime but little cemented	8.6
			Laveen sandy loam	
72	Buckeye-Beardsley Area, Ariz.	0–10	1. Grayish brown moderately to highly calcareous slightly nodular friable fine sandy loam	8.5
		10–40	2. Lime zone. Pinkish gray nodular slightly compact to compact heavy fine sandy loam to loam, slightly cemented	9.1
		40–56	3. Lime zone. Faint reddish brown, compact, highly calcareous, slightly lime-streaked, heavy fine sandy loam or loam	9.0
		56–72	4. Reddish brown, highly calcareous, compact fine sandy loam	8.9
			Laveen loam	
73	Salt River Valley Area, Ariz.	0–8	1. Dull reddish brown highly calcareous gritty loam	8.4
		8–22	2. Lime zone. Grayish brown compact light gritty loam slightly mottled with lime and carrying many lime concretions	8.4
		22–72	3. Lime zone. Gray to white moderately compact nodular limy clay loam, slightly cemented	8.4
			Moqui loam	
74	St. Johns Area, Ariz.	0–3	1. Reddish brown loam	8.4
		3–5	2. Gray-brown loam	8.7
		5–20	3. Compact zone. Reddish brown clay loam	8.1
		20–40	4. Lime zone. Grayish white silty clay loam, slightly cemented	8.5
		40–80	5. Reddish brown sands and gravels	8.2

TABLE 9—*Continued*

PRO-FILE NUM-BER	LOCATION	DEPTH *inches*	DESCRIPTION OF HORIZONS	pH
		Moqui sandy loam		
75	St. Johns Area, Ariz.	0–4	1. Reddish brown sandy loam	8.3
		4–5½	2. Grayish brown sandy loam	8.1
		5½–16	3. Compact zone. Reddish brown compact loam	8.1
		16–40	4. Lime zone. Grayish white sandy loam, slightly cemented	8.6
		40–80	5. Grayish brown sands and gravels	8.8
		Superstition sandy loam		
76	Yuma - Well- ton Area, Ariz. —Calif.	0–¼	1. Concentration of gravel, lime nodules, and loose sand	8.6
		¼–4	2. Slightly compacted light grayish brown sandy loam with a slightly pinkish tinge	8.6
		4–20	3. Lime zone. Somewhat compact light grayish brown to pinkish gray sandy loam, containing many gray lime nodules	8.3
		20–40	4. Lime zone. Very slightly compacted light grayish brown sand, containing many gray lime nodules	7.8
		40–72	5. Lime zone. Loose grayish brown sand, containing a few lime nodules	8.6
		Superstition sand		
77	Yuma - Well- ton Area, Ariz. — Calif.	0–6	1. Light pinkish brown slightly loamy firm sand	8.6
		6–30	2. Lime zone. Light pinkish grayish brown loamy sand, slightly compact, containing a large number of white lime nodules.	8.6
		30–72	3. Light grayish brown fine to medium loose sand	9.0

The four series studied are very uniform in the pH values of their profiles. In fact, the average pH value of all the horizons of all the profiles varies only 0.3 between the series (Mohave) having the highest average and the one (Superstition) having the lowest average. Of the 6 Mohave profiles 2 are mildly to strongly alkaline above the lime zone, and the other profiles are all strongly alkaline in their upper layers (tables 1 and 9). As in the sierozem and the desert soils, there appears to be no connection between cementation in the lime zone and the reaction of the profile, as the cemented horizons have virtually the same pH values as the uncemented horizons of that zone.

Five of the Mohave profiles have horizons above pH 8.5. In 1 profile every horizon is more alkaline than 8.5; in 1, highly alkaline layers occur in the compact zone; and in the 3 remaining profiles, the high pH is found in the lower part of the lime zone or in the parent material. In 2 profiles the compact zones have the highest pH values; in 1 profile the compact zone has a slightly higher pH than

the lime zone and the same as the surface horizon; in 1 it has the lowest pH in the profile; and in the 2 remaining profiles its reaction is intermediate between the horizon above and the lime zone.

All three Laveen profiles, as well as the profiles of the other red desert soils, are strongly alkaline above the lime zone. One profile has the same pH throughout. Two profiles have horizons more alkaline than pH 8.5. These occur in the lime zone in both cases.

Both of the Moqui profiles have horizons more alkaline than pH 8.5. In one profile this high alkalinity occurs next to the surface layer and just above the compact zone; in the other, it occurs in the lime zone and in the parent material. In both profiles, the pH values of the compact zones are the lowest, although in one the pH is the same as that of the horizon above it.

All of the horizons above the lime zone in both of the Superstition profiles are strongly alkaline (tables 1 and 9). The lowest pH (7.8) found in the desert and red desert soils occurs in the middle of the lime zone of one of these profiles (tables 8 and 9). Both profiles have horizons above pH 8.5. In one, these highly alkaline reactions occur throughout the profile, whereas in the other they occur in the surface horizons and in the lower part of the lime zone.

SUMMARY

Hydrogen-ion determinations were made in duplicate with a hydrogen electrode on samples of each of the horizons of several carefully selected profiles of a considerable number of important soil series in the United States. These series are classified into the various great soil groups. Every profile studied is located on a great soil group association map of the United States.

The lime zones of the pedocal profiles range from mildly alkaline to very strongly alkaline in reaction.

In the grassland pedocals, such as the chernozem, chestnut, reddish chestnut, brown, and reddish brown soils, the horizons above the lime zones have virtually the same reaction range—medium-acid to strongly alkaline.

In the desert and red desert profiles the horizons above the lime zones have virtually the same reactions as the lime zones.

In the sierozem profiles the horizons above the lime zones tend to be more alkaline than those of the grassland pedocals but not so alkaline as those of the two desert groups. They are neutral to strongly alkaline.

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DETERMINATION OF CERTAIN PHYSICAL PROPERTIES OF FOREST SOILS: I. METHODS UTILIZING SAMPLES COLLECTED IN METAL CYLINDERS

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Pore volume, air capacity, water-holding capacity, and volume weight are now generally recognized by forest ecologists as important soil properties. Data concerning these characteristics are frequently useful in evaluating site quality and as a basis for judging the influence of stand composition, cultural treatments, reproduction methods, fire, or grazing on the soil.

Various methods are available for investigating these physical properties. The technique most widely used is perhaps that of Burger,[1] with or without modification, which employs metal cylinders of 1000-cc. capacity to obtain samples of undisturbed soil. Essentially the same technique as outlined by Burger has been used by the writer in forest soil investigations for a number of years. During this time several modifications have been developed which appear sufficiently advantageous to merit presentation.

MOISTURE CONTENT OF SOIL AT TIME OF SAMPLING

One of the first considerations when sampling with cylinders relates to the moisture content of the soil at the time the samples are taken. Many soils, particularly those which are fine textured, undergo appreciable change in volume with change in moisture content. Despite this well-known fact, investigators commonly sample soils without regard to their moisture status. Various workers have noted that samples of relatively dry soil may show a considerable amount of expansion when such samples subsequently saturated. The soil which thus expands beyond the cylinder rims is usually cut off and discarded.

In order to eliminate the problem created by expansion of soil samples taken in cylinders, and for a further more important reason to be discussed later, the writer has adopted the practice of sampling only when the soils are at their field capacity. This allows for full expansion of the soil under natural conditions prior to sampling and consequently obviates the questionable practice of removing and discarding soil which has expanded beyond the cylinder rims.

It must be admitted that the decision to restrict cylinder sampling to soils at their field capacity in order to obtain more acceptable values for volume weight is based mainly on theoretical considerations; experimental data have not been obtained by the writer. The only comparative measurements available seem to be those reported by Burger from Switzerland. He sampled two areas: one supporting an old forest of beech with occasional spruce and fir; and the other,

[1] Burger, H. 1923 Physikalische Eigenschaften der Wald- und Freilandböden. Bühler Buchdruck, Zürich.

a nearby area supporting a 10-year-old plantation stand. The soil in both stands was a marly loam. In each of the areas two sets of three cylinder samples (1000 cc.) were taken.[2] In the first set each cylinder was driven in to almost its full depth (11.4 cm.) and a headpiece of 4-liter capacity was attached to the top. Four liters of water was then poured in and after it had entered the soil the headpiece was removed, and, if necessary, the cylinder was driven farther in until exactly filled. The top was then closed with a cover and after 20 minutes the filled cylinder was removed, the soil cut off flush with the lower rim, and a cover applied thereto. The cylinder with sample was then weighed. In the second set of samples the cylinders were driven into the soil until exactly filled and after removal the ends were covered; no water was added. In the laboratory all cylinder samples were immersed in a tank of water for 24 hours to accomplish complete saturation. The soil which had expanded beyond the cylinder rims was cut off and discarded.

Burger's data indicate for the old forest area an average volume weight of 1.032 for the samples that were saturated in place, and 1.119 for the samples that were saturated in the laboratory (with removal of the soil which expanded beyond the cylinder rims). In the samples from the young plantation the comparable values were 1.074 and 1.130. Thus, the samples which expanded during saturation in the laboratory had a density 5.2 to 8.4 per cent greater than samples of soil which were saturated in place in the field. These differences, applying as they do to a soil which was characterized as a marly loam, should not be regarded as maxima which might be obtained.

LABORATORY PROCEDURE

In order to facilitate presentation of the laboratory procedures, two schedules of treatment, A and B, are recognized.

Schedule A

1. The cylinder with contained soil at field capacity (plus covers when these are used) is weighed, either in the field or in the laboratory. The total weight (Wt) less the weight of the cylinder (Wcy) and covers (Wc) represents the weight of the soil sample at field capacity, here designated as $Wsfc$.

$$Wt - Wcy - Wc = Wsfc$$

2. In the laboratory a filter paper and wire screen are applied to the lower end of the cylinder, and the whole is immersed in a tank of water for 24 to 48 hours. It is a better procedure to place the cylinder samples in an empty tank, then flow in water until it stands at about one-third of the cylinder height. After an hour water is again run into the tank, bringing the level to two-thirds the cylinder height; finally, after another hour, the water-level is brought to the top

[2] A third set of three samples was also taken from each of the two areas, but these are not considered in the present discussion. The procedure was the same as described for the second set above, except that the soil which expanded when the samples were saturated *was not removed.*

of the cylinder, where it is allowed to stand for 24 to 48 hours. By gradually raising the water level as suggested, it is presumed that better replacement of the air in the soil mass is obtained.

3. After remaining in water for 24 to 48 hours, the cylinder sample should contain no air, but actually complete replacement of air in cylinder samples by this treatment is impossible. At this stage, the investigator has the choice of two procedures, both of which give essentially the same result, as is shown by the data in table 1.

The first procedure, involving use of watertight covers, has been rather widely used in the past. It requires the application of tight covers to the cylinder ends while the cylinder is still under water. The tightly closed cylinder is then removed from the tank, quickly dried to remove surface water, and weighed in air. From the total weight of the system (cylinder + covers + soil + water) the pore volume may be readily obtained. Pore volume (in cubic centimeters) is taken to be equal to the weight of water (in grams) in the system and may be converted to a percentage value by the formula:

$$\frac{\text{Pore volume, cc.}}{\text{Volume of cylinder, cc.}} \times 100$$

The second procedure, involving weighing the cylinder and sample under water, is less troublesome and more rapid. An ordinary heavy-duty balance adapted for specific gravity measurements is used for weighing. The weight of water displaced by the soil is obtained by subtracting the weight of the soil under water from the weight of the dry soil in air. Pore volume in per cent is then obtained by the formula:

$$\frac{\text{Volume of cylinder, cc.} - \text{volume of water displaced by soil, cc.}}{\text{Volume of cylinder, cc.}} \times 100$$

The data in table 1 were obtained from 22 cylinder samples of Merrimac sand, each of which was examined by both of the foregoing procedures. It may be seen that the mean difference between results for the two methods amounted to only 0.54 ± 0.12 per cent, the values obtained by weighing under water being higher than those obtained when watertight covers were employed. The difference in values obtained by the two procedures is statistically significant, but the mean difference is so small as to be of no practical importance. It should be borne in mind that pore volumes obtained by either of these two procedures are only approximate, being based on the erroneous assumption that all air in the soil has been replaced by water after the 24–48-hour immersion. Of the two techniques, however, that involving weighing the sample under water is preferable.

4. This step is ignored if the soil was at its field capacity when the samples were taken, as recommended in step 1. As many investigators do not start with the soil at field capacity, however, it is necessary for them to obtain in the laboratory some measure of water-holding capacity. This may be accomplished

as follows: After the cylinder sample (with all the air presumably replaced by water) has been weighed, following either of the two procedures mentioned in step 3, it is set aside to drain. The objective is to determine the water-holding capacity, or the amount of water held by the soil against the force of gravity. Gravitational water which drains from the sample is taken as a measure of the air capacity, or the noncapillary pore space. Laboratory determination of the amount of water held by a soil sample against gravitational forces presents several problems; the values so obtained appear to exceed field capacity values. Two considerations are of prime importance, (a) the conditions under which drainage occurs, and (b) the time of drainage. Conditions under which drainage is accomplished vary greatly among different investigators. Some place the cylinder with contained soil on an inclined screen for drainage; others place the

TABLE 1

Comparison of values for pore volume obtained on cylinder samples of soil by two different methods

SAMPLE DESIGNATION	PORE VOLUME BY METHOD INVOLVING USE OF WATER-TIGHT COVERS	PORE VOLUME BY METHOD INVOLVING WEIGHING CYLINDER AND SOIL IN WATER	SAMPLE DESIGNATION	PORE VOLUME BY METHOD INVOLVING USE OF WATER-TIGHT COVERS	PORE VOLUME BY METHOD INVOLVING WEIGHING CYLINDER AND SOIL IN WATER
	per cent	*per cent*		*per cent*	*per cent*
1A	45.7	45.6	2BN	48.3	48.9
5B	38.9	39.1	2AN	55.0	55.1
12A	45.8	45.8	3BN	45.5	45.4
12B	44.4	45.4	4AN	53.7	54.8
13A	46.6	46.2	4BN	48.9	49.9
13B	41.8	41.7	8AN	61.4	61.8
14A	47.5	47.2	8BN	47.4	48.4
14B	37.3	38.1	9AN	49.2	49.8
1AN	56.0	57.1	9BN	47.9	48.5
1BN	49.4	50.9	13AN	49.5	51.1
2AN	57.3	58.3	13BN	48.4	48.7

cylinders on sand-flats. In either case the upper surface of the soil sample is usually protected against evaporation by a moist cloth. A fixed drainage time of 1 or 2 hours is commonly employed. Drainage of cylinder samples on screens has been found unsatisfactory, because of interface phenomena; it is better practice to accomplish drainage on sand-flats. A drainage period of 1 hour was used in earlier work. A 2-hour period was employed in the investigation presented here, but even this time appears to be too short.

In table 2 are presented data to illustrate the difference in water-holding capacity values (volume basis) obtained in the laboratory by the foregoing technique and in the field as described earlier in step 1. The values given were obtained by examination of each sample by both methods. Values for water-holding capacity based on the laboratory determinations averaged 22.5 ± 0.69 per cent higher than values based on field capacity. This difference is statistically significant at the 0.01 per cent level.

In view of the difficulty of determining the water-holding capacity of soil samples in the laboratory, there are distinct advantages to starting with the soil at its field capacity. When this practice is followed, drainage of gravitational water from the soil body occurs under natural field conditions which cannot be reproduced in the laboratory.

Following determination of weight after drainage in the laboratory (or after the pore volume is obtained if water-holding capacity is to be based on the moisture content of the soil at its field capacity), the soil sample is oven-dried to constant weight at a temperature of 105–110°C.

TABLE 2

Comparison of values for water-holding capacity obtained on cylinder samples of soil by two different methods

STATION	WATER-HOLDING CAPACITY, LABORATORY DETERMINATION; DRAINAGE ON SAND-FLAT FOR 2-HOUR PERIOD	WATER-HOLDING CAPACITY (FIELD CAPACITY); SOIL AT FIELD CAPACITY AT TIME OF SAMPLING
	per cent volume	*per cent volume*
1	39.7	20.8
2	47.2	25.6
3	43.5	20.3
4	56.2	33.2
5	35.9	16.9
6	46.3	25.9
7	45.5	22.0
8	42.7	21.4
9	50.2	25.1
10	44.0	20.4
11	43.9	20.4
12	49.1	22.0

5. Computation of results for the various physical properties may now be completed.

Pore volume (percentage volume):

$$\frac{\text{Pore volume, cc.}}{\text{Volume of cylinder, cc.}} \times 100$$

or

$$\frac{\text{Volume of cylinder, cc.} - \text{volume of water displaced by soil, cc.}}{\text{Volume of cylinder, cc.}} \times 100$$

Volume weight:

$$\frac{\text{Oven-dry weight of sample, gm. (step 4)}}{\text{Volume of cylinder, cc.}}$$

Water-holding capacity (percentage volume):

$$\frac{\text{Wsfc. gm. (step 1)} - \text{oven-dry weight of sample, gm. (step 4)}}{\text{Volume of cylinder, cc.}}$$

Air capacity (percentage volume):

$$\frac{\text{Pore volume, cc. (step 3)} - \text{volume of water (cc.) in sample when at field capacity}}{\text{Volume of cylinder, cc.}} \times 100$$

Specific gravity of soil material:

$$\frac{\text{Oven-dry weight of soil, gm.}}{\text{Volume of cylinder, cc.} - \text{pore volume, cc.}}$$

or

$$\frac{\text{Oven-dry weight of soil, gm.}}{\text{Volume of water displaced by soil, cc. (step 3)}}$$

Schedule B

1. Same as in schedule A. After determining the weight of the sample at field capacity the investigator may elect to remove the sample from the cylinder and allow it to dry as explained in the following section, "Effect of allowing samples to dry before determining pore volume." By drying the sample two advantages are gained: (*a*) determination of pore volume then may be delayed until a convenient time, and (*b*) step 5 may be eliminated, except for measurement of the temperature of the water in the pycnometer. It should be borne in mind that if step 5 is to be eliminated the *oven-dry weight of the sample must be established before proceeding with step 2*.

2. In the laboratory the entire sample is removed from the cylinder, placed in a vessel, and covered with water. It is allowed to stand, with occasional stirring for 24 to 48 hours.

3. The soil and water (at room temperature) are then transferred to a Pyrex desiccator, 8 inches in diameter, with a tubulated cover and subjected to a vacuum equal to 15 inches of mercury for 2 to 3 hours with occasional shaking. This treatment is designed to remove the air from the soil material. Two other treatments to remove the air from the soil (cold water without evacuation, and boiling for 2 hours followed by evacuation for 2 to 3 hours) were also tested. The results are presented in table 3. Pore-volume values by the cold-water treatment alone averaged 2.0 ± 0.32 per cent less than values derived from samples which were boiled 2 hours and evacuated 2 to 3 hours. This difference is statistically significant at the 0.01 per cent level. The soil employed in all these determinations was Merrimac sand; larger differences would probably result in fine-textured soils. Pore-volume values of samples that were boiled 2 hours and evacuated 2 to 3 hours averaged 0.3 ± 0.07 per cent greater than values obtained by evacuation in cold water. This difference, although small, proved to be statistically significant at the 0.01 per cent level.

It is generally recognized that incomplete removal of air from soil samples results in pore-volume values which are too low. Consequently, the treatment with cold water alone gave the least acceptable values, and the treatment involving boiling with subsequent evacuation gave the best results. There is actually very little difference, however, in results obtained by evacuation in cold

water and by boiling with subsequent evacuation. Of these two methods. evacuation in cold water is favored by the writer because of its greater simplicity,

4. Following evacuation of the sample in cold water a glass tube approximately 35 cm. long with a diameter of about 10 mm. and graduated in 0.1 cc. is then fitted in the tubulure of the desiccator cover; hereafter this apparatus is referred to as a pycnometer. Water is added until the level is brought to a predetermined reference point on the graduated glass tube. Care is taken not to trap any air bubbles in the pycnometer. The entire system, pycnometer, soil, and

TABLE 3

Comparison of values for pore volume obtained on soil samples by three different treatments (pycnometer determinations)

SAMPLE NUMBER	PORE VOLUME		
	Cold water with occasional stirring	Cold water with evacuation (15″ Hg)	Boiling with evacuation (15″ Hg)
	per cent	*per cent*	*per cent*
3A	44.7	47.6	47.9
7A	46.6	46.9	47.4
7B	41.9	42.5	42.9
10A	46.6	46.6	47.1
10B	44.3	45.4	45.6
11A	48.4	50.3	50.6
11B	47.9	47.7	47.9
12A	50.3	50.2	50.7
12B	47.1	48.8	48.5
13A	48.0	49.9	50.1
13B	44.8	46.5	46.9
14A	49.9	52.2	51.6
14B	41.7	44.3	44.8
1AN	55.9	59.5	59.9
1BN	50.1	51.4	51.9
2AN	56.3	61.0	61.7
2BN	49.7	50.8	50.9
3AN	53.1	57.0	57.7
3BN	49.0	50.7	50.9
4AN	55.8	56.9	57.2

water, is then weighed to the nearest gram on a heavy-duty balance having a capacity of about 25 kgm.

5. Sufficient water is then siphoned from the pycnometer to enable removal of the cover, and the temperature of the soil-water suspension is taken. (If the oven-dry weight of the sample has been previously established, the remaining procedure in this step becomes unnecessary.) The water remaining in the pycnometer is decanted and added to that previously siphoned off. The combined soil-water suspension is placed aside to settle for about an hour.

Most of the soil remains in the pycnometer and this is now washed, with as small a volume of water as possible, into a pan or other vessel for drying. Initial drying is accomplished on a hot plate followed by final drying in an oven.

The aforementioned soil-water suspension is carefully decanted so as to retain the coarser soil fraction in the vessel; this latter material is next washed into a beaker of about 600-cc. capacity, taken down to dryness, and weighed.

The volume of the remaining soil-water suspension (containing clay and some silt) is determined and, after thorough stirring, two 200-cc. aliquots are removed. These aliquots are placed in beakers of about 250-cc. capacity, taken down to dryness, and weighed.

With the foregoing data it is a simple matter to arrive at the oven-dry weight of the entire soil sample.

6. It is necessary to establish the weight of the pycnometer when filled with water at the same temperature as that recorded in step 5. A convenient method is to obtain measurements of weight of the water-filled system for several temperatures and from these derive a curve. From this curve the weight corresponding to any desired temperature may be obtained.

7. Computation of volume-weight and pore-volume values may be most readily understood by reference to the following form which has been used in recording the various measurements. For convenience the column headings have been designated by Roman numerals. If the dry weight of the entire sample was established in step 1, the value is entered in column X, and columns IV–IX are omitted.

    I. Sample number or designation.
   II. Weight of the system: pycnometer + soil + water, gm.
  III. Temperature of water in II.
  IV. Volume of soil-water suspension (containing clay and silt), cc.
   V. Duplicate aliquots of IV (normally 200 cc. each).
  VI. Weight of sediment in V (both aliquots), gm.
 VII. Total weight of sediment in IV based on amount in aliquots VI, gm.
VIII. Weight of sediment which settled out during the 1-hour period, gm.
  IX. Weight of the main soil sample, gm.
   X. Dry weight of the entire soil sample (VII + VIII + IX) gm.
  XI. *Volume weight:* X ÷ volume of sample of soil in place, i.e., volume of cylinder, cc.
 XII. Weight of water displaced by soil sample (weight in gm. of pycnometer filled with water at the same temperature as in III + X) − II gm.
XIII. Volume of water displaced by soil (XII corrected for density at the temperature observed), cc.
XIV. *Pore volume, per cent:*

$$\frac{\text{Volume of soil in place, cc.} - \text{XIII}}{\text{Volume of soil in place, cc.}} \times 100$$

8. Computation of water-holding capacity, air capacity, and true specific gravity is the same as in schedule A.

COMPARISON OF RESULTS OBTAINED IN SCHEDULES A AND B

Water-holding capacity

If water-holding capacity is based on the moisture content of soil in place at its field capacity the result is the same in both schedules. If, however, the water-holding capacity is based on a laboratory determination (immersion of the

sample in water for 24 to 48 hours followed by drainage on a sand-flat for 2 hours), values averaging 22.5 per cent (volume basis) too high are obtained as indicated in table 2.

Pore volume

Comparisons of pore-volume results from three different treatments in the pycnometer have been considered in table 3. It remains to compare these results with those obtained by the technique mentioned in schedule A, step 3 (immersion in a tank of water for 24 to 48 hours followed by weighing under water).

On the basis of 20 cylinder samples the latter method gave results which averaged 4.7 ± 0.31 per cent lower than were obtained for the same samples

TABLE 4

Comparison of results for volume weight of cylinder samples of soil by two different methods

SAMPLE NUMBER	VOLUME WEIGHT		SAMPLE NUMBER	VOLUME WEIGHT	
	Treatment as in Schedule A	Treatment as in Schedule B		Treatment as in Schedule A	Treatment as in Schedule B
3A	1.371	1.369	13B	1.398	1.394
7A	1.400	1.395	14A	1.247	1.249
7B	1.516	1.516	14B	1.467	1.467
10A	1.397	1.395	1AN	1.023	1.017
10B	1.451	1.453	1BN	1.252	1.248
11A	1.291	1.291	2AN	1.011	0.996
11B	1.355	1.359	2BN	1.305	1.300
12A	1.273	1.263	3AN	1.096	1.096
12B	1.352	1.351	3BN	1.299	1.298
13A	1.284	1.281	4AN	1.131	1.129

after boiling and evacuation in the pycnometer. This difference is significant at the 0.01 per cent level.

It may also be pointed out that the technique described in schedule A, step 3 results in pore-volume values (based on 20 samples) 2.9 ± 0.43 per cent lower than those obtained by pycnometer measurements with cold water without evacuation. Presumably these differences are greater in fine-textured soils because of the greater difficulty of removing the contained air.

Volume weight

If the soil is at its field capacity when sampled one would expect that volume-weight values obtained in schedules A and B would be the same. That this is essentially true is indicated by the data in table 4. Analysis of these data show that volume weight as determined in schedule A averages 0.0026 ± 0.0009 higher than that by the treatment (boiling with subsequent evacuation) in schedule B. This difference, although very small, is statistically significant at the 0.01 per cent level. Presumably the difference results from losses of soil

material in boiling or handling.   From a practical point of view the differences
are negligible.

Air capacity

Air-capacity values are obviously influenced by the values for both pore
volume and water-holding capacity.   If pore-volume values are too low the air-
capacity values will be correspondingly too low.   On the other hand, if water-
holding capacity values are too high the air-capacity values will be correspond-
ingly too low.

True specific gravity of soil material

Incomplete removal of air from the soil sample is a principal source of error
in determinations of true specific gravity.   If air is incompletely removed, the

TABLE 5

*Comparison of results for true specific gravity of cylinder samples of soil by two
different methods*

SAMPLE NUMBER	SPECIFIC GRAVITY		SAMPLE NUMBER	SPECIFIC GRAVITY	
	Treatment as in Schedule A	Treatment as in Schedule B		Treatment as in Schedule A	Treatment as in Schedule B
3A	2.38	2.61	13B	2.40	2.60
7A	2.37	2.63	14A	2.38	2.61
7B	2.39	2.64	14B	2.34	2.63
10A	2.37	2.61	1AN	2.32	2.51
10B	2.45	2.66	1BN	2.47	2.57
11A	2.34	2.60	2AN	2.37	2.55
11B	2.34	2.60	2BN	2.52	2.64
12A	2.35	2.54	3AN	2.44	2.55
12B	2.43	2.64	3BN	2.38	2.63
13A	2.40	2.56	4AN	2.44	2.62

reported volume of the solid soil material is too high and the true specific gravity
consequently too low.   This is indicated by the data in table 5.

True specific gravity values obtained in schedule A (cylinder samples im-
mersed in water for 24 to 48 hours) average 0.21 ± 0.01 lower than values ob-
tained in schedule B (evacuation in cold water for 2 to 3 hours).   This difference
is statistically significant at the 0.01 per cent level.   Values for true specific
gravity based on pycnometer measurements without evacuation or boiling fall
between those presented in table 5.

EFFECT OF ALLOWING SAMPLES TO DRY BEFORE DETERMINING PORE VOLUME

Sometimes, because of lack of laboratory facilities near the point where field
work is being conducted, it is impossible or inconvenient immediately to proceed
beyond step 1 in schedule B.   The question then arises whether the sample may
be removed from the cylinder, dried, and stored until it can be returned to a

central laboratory or until it is convenient to complete the examination.   Consideration has been given this question.

It is obvious that values for volume weight and water-holding capacity will not be affected, but it seemed possible that drying might cause changes which would alter the values for pore volume and air capacity.   Tests were conducted with 10 samples to supply information on this point.   Pore volume of each sample was first determined by the procedure described in schedule B (evacuation in cold water for 2 to 3 hours).   The full sample was then recovered in each case and after being oven-dried and weighed was again allowed to soak in water for 24 to 48 hours.   Again pore volume was determined by the procedure indicated above.   Results of these tests are presented in table 6.   Pore-volume values for the samples which had been oven-dried averaged 0.1 ± 0.14 per cent higher than values for samples not oven-dried.   This difference is not statistically

TABLE 6

Comparison of pore-volume values determined for samples of soil in their field condition and after being oven-dried (samples in cold water in pycnometer plus evacuation)

STATION	PORE VOLUME, BASED ON FIELD CONDITION	PORE VOLUME, BASED ON OVEN-DRIED SAMPLES SUBSEQUENTLY SOAKED 24 TO 48 HOURS
	per cent	*per cent*
2A	48.7	48.8
4A	49.7	49.1
6A	46.6	46.4
9A	49.1	48.7
10A	46.4	46.5
1AN	59.9	60.1
2AN	60.4	60.9
3AN	60.5	60.8
4AN	56.7	57.6
6AN	61.1	61.1

significant, indicating that pore-volume values obtained by either procedure are equally satisfactory.   Since pore volume does not undergo any significant change as a result of drying of the samples, it follows that air-capacity values, likewise, will undergo no change.

This finding also suggests that an important saving of time can be effected in schedule B.   Preceding step 2 in schedule B the soil may be removed from the cylinder, oven-dried, and *weighed*.

With the oven-dry weight of the sample thus established it will be obvious that except for the measurement of water temperature, step 5 becomes unnecessary and may be eliminated.

SUMMARY AND CONCLUSIONS

Pore volume, air capacity, water-holding capacity, and volume weight are conveniently measured in samples of undisturbed soil collected in metal cylinders.

Whenever possible the moisture content of the soil should be at field capacity

when sampling is carried out. This provision eliminates problems arising as a result of expansion of fine-textured soils that are sampled in dry condition and subsequently wetted. A further, more important advantage is that drainage of gravitational water from the soil body occurs under natural conditions, with the result that the moisture content at field capacity is a more acceptable measure of water-holding capacity than can be obtained in the laboratory. In Merrimac sandy loam, water-holding capacity determined in the laboratory (samples in cylinders submerged in water for 24 to 48 hours followed by drainage for 2 hours on a sand-flat) exceeded the field capacity by an average value of 22.5 ± 0.69 per cent (volume basis).

Two schedules of procedure are presented for measuring the physical properties of soil samples collected in metal cylinders. Assuming that the soil to be investigated is at its field moisture capacity, and this initial condition is recommended, both schedules will result in essentially the same values for field capacity (here taken as equivalent to water-holding capacity) and volume weight. Values for pore volume will be highest, and presumably most accurate, when the samples are treated according to one of the procedures in schedule B (sample removed from cylinder and allowed to stand in water for 24 to 48 hours, followed by boiling for 2 hours and finally evacuation for 2 to 3 hours). This treatment is very time-consuming and for practical purposes results in values nearly the same as those obtained by the preferred procedure in schedule B (as above but the samples are not boiled). Samples receiving the latter treatment averaged only 0.3 ± 0.07 per cent lower in pore volume than samples receiving the treatment involving boiling. In the third treatment tested in schedule B the samples were neither boiled nor evacuated, but as in the other schedule B treatments the volume of the soil material was determined in a pycnometer. Pore volume determined by this third treatment averaged 2.0 ± 0.32 per cent less than by the procedure involving both boiling and evacuation. Results obtained by the procedure in schedule A (cylinders with contained samples allowed to stand in water 24 to 48 hours and then weighed under water) indicate pore-volume values averaging 4.7 ± 0.31 per cent less than values obtained in schedule B (both boiling and evacuation). As pointed out earlier, incomplete removal of air from the soil sample results in low values for pore volume.

Air-capacity values bear an intimate relationship to pore-volume values. If the latter are too low, the former will be correspondingly too low. On the other hand, if values for water-holding capacity are too high, air-capacity values will be correspondingly too low.

Values for true specific gravity are too low if all air is not removed from the soil sample. This explains why true specific gravity determined according to the procedure in schedule A averages 0.21 ± 0.01 lower than values obtained in schedule B (evacuation for 2 to 3 hours).

If samples are to be investigated by the procedure in schedule B, they may be removed from the cylinders after being weighed to determine the moisture content at field capacity. These samples may then be dried and stored until it

is convenient to complete the determinations. No statistically significant difference in pore volume could be established between samples which were investigated in their field condition and those which had been oven-dried. This fact is of much practical importance for investigators who may be working far from laboratory facilities. Furthermore, by establishing the oven-dry weight of the sample prior to proceeding with step 2 in schedule B, it is possible to eliminate the time-consuming technique of step 5.

AUTHOR INDEX

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